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Colon Cancer Diagnosis and Therapy

Volume 2

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This book is dedicated to our families, our teachers, and friends

Preface

Colorectal cancer (CRC), a heterogenous disease, is the third most commonly diagnosed cancer. As it is highly malignant, it is the second leading cause for cancer-related mortalities. As estimated by the American Cancer Society for the year 2020, 43,340 rectal cancer cases and 104,610 colon cancer cases were diagnosed. The mortality rate estimated for CRC was 53,200 for this year. The conventional therapy is surgery followed by radiation and chemotherapy. However, recurrence after surgery and therapies is a major obstacle that results in metastatic stages. In general, the 5-year survival rate is always considered a hurdle due to lack of early diagnostic tests as well as disease recurrence following treatment. Additionally, both modifiable and non-modifiable risk factors are primary causes for the occurrence of CRC. Modifiable risk factors include being physically inactive, overweight, smoking, and consumption of alcohol, while the unmodifiable risk factors are age and inherited syndromes (HNPCC and FAP). Thus, it is essential for the researchers and clinicians to develop novel therapeutic strategies to diagnose CRC at earlier stages via biomarkers. In this volume, our authors have included novel advanced diagnostic and therapeutic strategies for CRC treatment.

The common diagnostic options for CRC detection are colonoscopy and FOBT, while novel diagnostic strategies include molecular biomarkers to improve early detection of CRC. Our authors have described varied molecular techniques for diagnosis of CRC along with recent therapeutic strategies in this volume. The conventional therapeutic regimens include chemo and radiotherapy. Despite the many advances in research, patients often develop metastasis and recurrence. This is mainly attributed to the intratumoral heterogeneity nature of CRC. Hence, studying single cancer cell resolution at molecular level to understand the heterogeneity of the tumor is very much essential for therapy and prediction of CRC. Dariya et al. have described all essential applications of single cell technology in CRC. Additionally, inactivation of mismatch repair genes responsible for microsatellite instability are detected in almost 90% of HNPCC and 15% of sporadic CRC cases. The inactivation of these mismatch repair genes is due to the hypermethylation of promoter region of these genes. The epigenetic alterations likely promote escape of apoptosis of cancer cells. Thus, targeting these genes or identifying them

as biomarkers potentiates the diagnosis of CRC in its early stages. In our volume, Gupta et al. have described role of DNA mismatch repair genes in CRC progression. Furthermore, the research studies demonstrated that in addition to tumor microenvironment, extracellular vesicles play a major role in cell-cell communication. The extracellular vesicles are cell derived vesicles that possess lipids, proteins, mRNA, and small RNA. The extracellular vesicles secreted by the cancer cells play a crucial role in promoting metastasis. Thus, impairing biogenesis and secretions of extracellular vesicles by the cancer cells would potentiate the cancer therapy. In another way, they are also considered efficient cargo that can deliver therapeutic drugs to the target cells for cancer therapy. The extracellular vesicles also function as biomarkers and are included in diagnosis and prognosis for cancer metastasis. Thus, extracellular vesicles are promising tools for diagnosis and therapy. Merlin et al. have outlined the importance of extracellular vesicles in CRC growth, metastasis, diagnosis, and therapy. Moreover, targeting glycolysis is now an attractive clinical strategy in cancer diagnosis and therapy. This is due to the predominant nature of the cancer cells that produce energy by glycolysis followed by lactic acid fermentation even in the presence of oxygen, otherwise known as "Warburg Effect." Thus, glycolysis is important for carcinogenesis. Therefore, researchers are now focusing on targeting the inhibitors against the critical enzymes involved in glycolysis, which showed a promising anticancer effect. In this regard, Jaiswara et al. have described the emerging therapeutic approach of targeting the aerobic glycolysis in colon cancer cells. Similarly, targeting glutamine would be an effective approach to treat CRC cells, because glutamine is required as a supplement for cancer cells culturing and is utilized as fuel for various cycles like the tricarboxylic acid cycle. In this volume, our authors have outlined the applications of targeting glutamine metabolism in CRC. With advances in research, nanotechnology is now gaining global consideration due to its improved standards for diagnosis and therapy that avoid toxicity to the healthy cells. This nanotechnology includes engineering of novel organized materials capable for diagnosis and therapeutic approach. The nanoparticles included for CRC therapy were based for identification of tumor, cancer biomarkers, and drug delivery by using biologically targeted contrast agents. In this volume, Reshmitha et al. and Vijay et al. have outlined nanotechnology approach for CRC diagnosis and therapy, as well as recent advances in nanomedicine-based drug targeting.

The epidemiological and experimental findings from the past decades have determined that the dietary intake is associated with risk of CRC occurrence. This is due to the influence of diet that effects interacting mechanisms like inflammation, immune responsiveness and obesity. Thus, an alteration in the diet would therefore be a promising approach to reduce CRC incidence. In this volume, Shinde et al. and Kulshrestha et al. have outlined the dietary habits and associated risk for CRC occurrence and incidence. Additionally, in this volume are preventive effects on CRC with the use of Indian food. The Indian traditional cuisine ingredients are rich in preventive therapeutic strategies that aim to reduce the incidence and mortality rate of the patient. Moreover, the Indian herbals are highly rich in phytochemicals with varied medicinal properties including antioxidative, anticarcinogenic and anti-

mutagenic. Researchers are now widely focusing on combinational therapies for cancer treatment including phytochemicals along with chemodrugs. Phytochemicals may have the ability to sensitize the tumor cells to chemodrugs and reduce adverse effects. In this volume, Durgambica et al. have given the latest insight into therapeutic potentiality of phytochemicals against CRC. As an example, seaweeds, rich in terpenoids, polyphenols, polysaccharides, and steroids, are found to have promising pharmacological activities like cancer therapy. Pandey et al. have explored seaweeds as potential human colon cancer therapy. Similarly, Sandeep et al. also have described naturally available chemo-sensitizing and immunomodulatory agents in colon cancer.

The intestinal bacteria like *Helicobacter pylori* infects the gastro mucus epithelial layer. This results in peptic ulcers, gastritis, and eventually results cancerous diseases like CRC. Therefore, knowledge about the casual relationship and mechanism behind the risk for CRC occurrence from to *H. pylori* infection is essential. Our volume includes chapters describing the role of bacterial infection in colon carcinogenesis and the associated therapeutic approaches. Use of probiotics is now widely encouraged to exclude the pathogenic intestinal flora and reduce the carcinogenic secondary bile acids, thus preventing colon cancer. Although previous studies have shown that probiotics have an inhibitory effect on tumor development, it, however, remains unclear and proper investigation is necessary. In this volume, Anaga et al. included therapeutic approach of probiotics in colon cancer therapy.

Altogether, the present editorial book provides an in-depth understanding of novel diagnostic and therapeutic options currently available. Our authors have explored current advances included the applications for CRC diagnosis and therapy. It is our immense pleasure to present this comprehensive summary to the scientific community for the benefit of patient care.

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Naveen Kumar Vishvakarma is currently assistant professor of biotechnology at Guru Ghasidas Vishwavidyalaya. He earned his master's degree in microbiology and then did his doctoral research in tumor immunology. During his doctoral research, he worked in the area of tumor acidity–mediated immunosuppression. After completing doctoral research work, he worked as postdoctoral fellow/research associate at Banaras Hindu University, Manitoba Institute of Cell Biology (Canada), and Moffitt Cancer Center and Research institute (USA). During his work at Moffitt Cancer Center, he demonstrated the role of acidic tumor microenvironment in selection of aggressive phenotype with metabolic alterations. In 2013, he joined HNB Garhwal University as assistant professor and later moved to his current position at Guru Ghasidas Vishwavidyalaya in 2014. His current research interest includes modulation of tumor metabolism, evaluating derivative anticancer drugs, and chemosensitization.

Chapter 1
Application of Single Cell Technology in Colorectal Cancer

Begum Dariya and Ganji Purnachandra Nagaraju

Abstract Colorectal cancer (CRC) is the second most common malignant disease among the cancer-related deaths globally. The incidence rate and mortality rate over the past decade remain high with the reduced survival rate of the patient, despite many advanced conventional and research clinical practices. Therefore, there is an urgent requirement for novel screening methods to diagnose tumor at its earlier stages with the development of predictive markers and therapeutic tools. CRC is a complex heterogenous disease as patients respond differently to a single therapeutic regimen due to the intratumoral heterogeneity (ITH). Additionally, they cannot be diagnosed due to asymptomatic condition of the disease, which is dormant for longer periods. During CRC progression, premalignant cells show varied epigenetic alterations including histone modifications, chromatin alterations, DNA methylation, and nucleosome positioning that determine the phenotypic nature of CRC. The single cell sequencing technologies are widely encouraged to detect the single cell RNA transcriptome, single cell DNA genome, ITH, and multi-omics of a single cell taken from a tumor. In this chapter we have focused on varied sequencing techniques of genome and applications of single cell technologies in CRC. This would provide essential data for identifying novel prognostic biomarkers for personalized cancer therapy of the patient.

Keywords Colorectal cancer · Single cell sequencing · Intratumoral heterogeneity · DNA methylation · Histone modifications

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Abbreviations

CCDGs	Cell clustering deregulated genes
CCGA	Circulating cell-free genome atlas
CCSDGs	Cell cluster specifically deregulated genes
cfDNA	Circulating free DNA
CNVs	Copy number variants
CRC	Colorectal cancer
CTCs	Circulating tumor cells
ctDNA	Circulating tumor DNA
EMT	Epithelial-mesenchymal transition
FACS	Fluorescence-activated cell sorting
GO	Gene ontology
ITH	Intratumoral heterogeneity
KEGG	Kyoto Encyclopedia of Genes and Genomes
LCM	Laser capture microdissection
LINE-1	Long interspersed nuclear element 1
LTRs	Long terminal repeats
MET	Mesenchymal-epithelial transformation
MSI	Microsatellite instability
NGS	Next-generation sequencing
scChIC-seq	Single cell chromatin immune cleavage followed by sequencing
scChIL-seq	Single cell chromatin integration labelling followed by sequencing
scChIP-seq	Single cell chromatin immunoprecipitation with DNA sequencing
scCOOL-seq	Single cell multiple sequencing technique
scCUT&Tag	Single cell cleavage under targets and tagmentation
scDNA-seq	Single cell DNA sequencing
scRNA-seq	Single cell RNA sequencing
scRRBS-seq	Single cell reduced representation bisulfite sequencing
SCS	Single cell sequencing
scTHS-seq	Single cell transposon hypersensitive site sequencing
scTrio-seq	Single cell triple omics sequencing
snDROP-seq	Single-core sequencing
SNV	Single nucleotide variation
TCGA	The Cancer Genome Atlas
TSCS	Topographic single cell sequencing
WGA	Whole genome amplification

1.1 Introduction

Colorectal cancer (CRC) is a heterogenous disease and significant global health problem. It ranks third for the most commonly diagnosed cancers and is the second leading cause of cancer-related mortalities (Khazaei et al. 2019). As analyzed, almost 30–50% of CRC diagnosed progress into advanced metastatic stages with just 5 years of survival rate (Engstrand et al. 2018). Despite multiple advances in therapeutic options, metastasis always remains as a major threat for the effective therapies. Metastasis remains as a significant challenge for CRC therapy which is mainly attributed due to the intratumoral heterogeneity (ITH) and presence of circulating tumor cells (CTCs) (Séronie-Vivien 2014). The heterogenous nature of CRC is also related with accumulation of varied alterations or mutations of the regulatory genes and their associated pathways (Yanus et al. 2013). In addition to mutations, epigenetic modifications like histone modification, DNA hypermethylation, and microsatellite were also found to play crucial role in promoting CRC progression (Lao and Grady 2011). Thus, CRC is a complex disease at both genome and clinical manifestation levels.

Single cell sequencing (SCS) technologies are developed widely since last decades and are believed as an efficient tool for prognostic and diagnostic applications in the field of oncology. It provides unique means of characterizing ITH, analyzing CTCs and cancer stem cells, and detecting the genome of varied cancer cells to reconstruct or map the evolutionary lineage of tumor cells for better understanding in immuno-oncology, metastasis, and resistance against therapies (Tsoucas and Yuan 2017). Furthermore, SCS would promote possible ways to detect cancer progression at their early stages and potentiates in developing medicine. These SCS technologies include multiple methods for the study of single cell genome, single cell transcriptome, single cell epigenome, next-generation sequencing, and computational techniques for single cell data analysis. In this chapter we have summarized the applications of SCS technologies for the beneficiary of CRC therapy.

1.2 Isolation Methods for Single Cells and Recent Developments in Single Cell Techniques

The primary steps for SCS include isolation of viable cells through multiple isolating methods. There are several approaches currently encouraged such as laser capture microdissection (LCM) (Nichterwitz et al. 2016), microfluidics (Dittrich and Manz 2006), limiting dilution (Gross et al. 2015), fluorescence-activated cell sorting (FACS) (Shapiro et al. 2013), microwells (Tsaytler et al. 2011; Gierahn et al. 2017), micromanipulations (Guo et al. 2017), and in situ barcoding (Rosenberg et al. 2018; Cao et al. 2017). Additionally, 10x Genomics commercially offers chromium system for profiling single- cell transcriptomes. These approaches likely improve the resolution of SCS and promote the ability to investigate the rare cell

transitional states. Furthermore, the advances in scientific research proposed novel combinatorial approaches for single cell techniques. They include single cell whole genome amplification methods, single cell combinatorial marker sequencing technique (Vitak et al. 2017), single cell multiple sequencing technique (scCOOL-seq) (Guo et al. 2017), topographic single cell sequencing (TSCS) (Vickers 2017), and SPLit-seq technology (Rosenberg et al. 2018). The efficacy of SCS technology is further improved by combining with other technologies. For instance, CRISPR screening was combined with single cell RNA sequencing to develop CROP-seq (Datlinger et al. 2017; Vickers 2017). This improves the analysis of various heterogenous cell populations and determines the relationship between genes and regulatory elements. sNuc-seq was combined with microfluidic technology to develop single cell nuclear RNA sequencing (Habib et al. 2017). Similarly, single cell transposon hypersensitive site sequencing (scTHS-seq) and single-core sequencing (snDrop-seq) lay platform to detect nuclear transcripts and epigenetic features (Ruderfer et al. 2018). Thus, these SCS technologies are made advanced giving a technical base for constructing a comprehensive cell map and potentiating the single cell research. Plans are being made to combine the highly advanced multi-omics with SCS techniques in the future to study complex tissue for diagnosis and therapy of various clinical diseases.

1.2.1 Single Cell Genome (DNA) Sequencing

The genome sequencing of solitary cells or nuclei is well established that resolves intercellular variations present in the heterogenous cellular population isolated from a tissue to tumor (Gawad et al. 2016; Cheow et al. 2016). The whole genome amplification (WGA) of single cell genome is constantly essential for single cell DNA sequencing (scDNA-seq). Zhang et al. (Zhang et al. 2018) have investigated on amplification of CREPT gene using WGA in CRC. CREPT is a potential oncogene found upregulated in various cancer patients like CRC. It accelerates proliferation and metastasis in both in vitro and in vivo. The CREPT amplification detected from the primary CRC was about 48.28%, and its overexpression is correlated with poor survival. However, their RNA-sequencing analysis also uncovered the enhancing effect of CREPT that promotes the interaction between p300 and β-catenin that eventually activated Wnt/β-catenin pathway. Additionally, they also found that CREPT in association with p300 leads to the activation of histone acetylation markers including H3K27ac and H4Ac that reduces repressive histone marker H3Kme3 present at the promoter site of Wnt downstream target. Thus, CREPT can serve as a prognostic biomarker for CRC patients. Additionally, Leung et al. (2017) also performed single cell DNA sequencing using WGA. They traced the lineage of metastasis in two CRC patients having matched liver metastasis. In one patient, a monoclonal seeding was detected with a single clone evolving varied number of mutated genes prior to migration to the secondary site, the liver, while in the other patient, a polyclonal seeding was detected with two independent clones seeded with

metastatic liver diverged from the primary tumor. Indeed, in both the cases of CRC patients, driver mutation genes including APC, TP53, CDK4, KRAS, and NRAS were detected in organ sites of both primary and metastatic regions. Though WGA potentiates the sensitivity of scDNA-seq, the DNA sequencing targets on copy number variants (CNVs) for accurate amplification of single nucleotide variations (SNVs) as high sensitivity is essential. The SNVs detected for CRC showed seven SNVs and are shared in more than one tumor. Additionally, the intermittently found novel genes includes ZNF717, ARMC4, ZNF493, SUMF2, CDR1 and gains in 10q25.3 are mutated in CRC. Thus, about 99% of analyzed SNVs were private events with high frequency that confirms the increased heterogenous mutations in CRC (Liang et al. 2019).

1.2.2 Single Cell Transcriptome (RNA) Sequencing

The single cell transcriptome sequencing or single cell RNA sequencing (scRNA-seq) is used to measure the expression of gene/mRNA of a single cell for better understanding about the tumor heterogeneity from the bulk of tumor cell population (Sadanandam et al. 2014). The first study was performed by Li et al. (2017) wherein their study involved in sequencing single cell RNA taking samples from 11 CRC patients analyzed 969 resected primary tumor cells and matched with seven patients having normal mucosa for 622 single cells. They used a novel clustering method called reference component analysis and developed seven clusters. The seven clusters marked are B cells, T cells, myeloid cells, mast cells, epithelial cells, endothelial cells, and fibroblast cells. Additionally, they also detected upregulated EMT-related genes in CAF samples of CRC. Similarly, Dai et al. (2019) also performed scRNA-seq to profile cells of CRC patient using Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analysis. They analyzed 2824 cells and differentiated the CRC tissue into five distinct clusters via scRNA-seq. The GO analysis determines that each cluster exhibits diverse functions. About three clusters are found related with energy transport and generation of extracellular matrix, while the other two clusters are involved in immunology process. Thus, this provides better understanding of heterogeneity of CRC. Similarly, the single cell transcriptome analysis can also be done in a study to characterize the CRC liver metastasis taken from the tumor immune microenvironment (Zhang et al. 2020). The ScRNA-seq was performed to compare between metastasis CRC tissue and adjacent tissue from the patient. Later, clustering was done differentiating the sample into 12 clusters in accordance to 6 cell types including tumor cells, B cells, myeloid cells, T cells, fibroblast cells, and endothelial cells. The expression clustering of 445 cell clustering deregulated genes (CCDGs) detected 6 gene modules and are analyzed for functional enrichment. The pathways involved in angiogenesis, immune response, and cell-cell adhesion in the cancer cells along with the T-cell population were identified. The cell cluster specifically deregulated genes (CCSDGs) for the expression of 93 cells were performed in tumor-infiltrating immune cells analyzed with TCGA

dataset in relation to the survival rate of the patients. In this regard the deregulated genes correlated were identified as SPP1, SPARCL1, GPNMB, and MGP. Similarly, genes that are responsible for the inflammatory response including CXCL8, CCL18, CCL3, CCL18, CCL4, and CXCL3 showed significantly higher expression. Additionally, Wnt signalling pathway that induced migration of granulocytes was also found activated. Thus, this novel dataset identification that includes differentiation of immune cells and dysregulated pathways of the involved granulocytes in TME advances the diagnosis and therapy of mCRC.

1.2.3 Single Cell Epigenome Sequencing: DNA Methylation and Histone Sequencing

Epigenetic modifications including DNA methylation, microsatellite instability, and histone modifications play crucial role in the development and progression of CRC (Lao and Grady 2011). These epigenetic modifications in genes also serve as targets in chemotherapy. For instance, from an epigenome-wide study, five potential genes including PON3, CHL1, CCNEI, DDX43, and CCNBP1 are found to be highly expressed in CRC recurrence. These five genes served as therapeutic targets for individual patients that developed resistance against 5-FU (Baharudin et al. 2017). The gene expression is also regulated by the modifications in chromatin structure and epigenetic markers that induce heterogeneity responsible for varied disease occurrence (Margueron and Reinberg 2010). The DNA methylation at the CG-rich sites is correlated with transcriptional silencing. The DNA methylation can be studied at the single cell level via various technologies including restriction enzymes sensitive to methylation and bisulfite conversion. The bisulfite sequencing for the methylated DNA loci sites can be inferred from PCR-amplified sequencing. The advanced version for bisulfite includes single cell reduced representation bisulfite sequencing (scRRBS-seq) (Guo et al. 2015). This sequencing aids for identification of about 1.5 million CpG sites in a genome of a single cell. Similarly, Lynch syndrome was screened to develop a tube screening panel. The screening was done for a comparative test that includes microsatellite instability (MSI), BRAF mutation, and MLH1 methylation promoter. The primers are used in PCR for MLH1 promoter for methylation and mutation in BRAD. MSI was also tested comparing five nucleotide markers including EWSR1, BAT26, MYB, BAT25, and BCAT25. This screening included a blind screening of 12 cases. Among them, five cases are identified as MSS, three cases as MSI/possible Lynch syndrome, and four cases as MSI/non-Lynch syndrome (Dehghanizadeh et al. 2018). Similarly, in another study, sessile serrated polyps were characterized for genetic and epigenetic landscapes. These polyps are significant precursors for sporadic CRC, and detecting these polyps via colonoscopy is highly critical. Therefore, to analyze the samples of serrated polyps, a combination of profiling is done for genome-wide mutation detection, DNA methylation, and exome sequencing. The profiling was performed using methyl array and bisulfite sequencing for whole genome. The analysis from the study revealed

that BRAF-V600E was responsible for the recurrence of somatic mutations in sessile serrated polyps and is associated with DNA methylation (Dehghanizadeh et al. 2018). DNA methylation profiles from varied studies were also analyzed to detect specific markers that respond to chemotherapy. TFAP2E (transcription factor AP-2 epsilon) is a protein detected as clinically unresponsive to various drugs like 5-FU and oxaliplatin. Additionally, the hypermethylated TFAP2E is also responsible for the development of chemoresistance and is associated with mutation in BRAF over the dysregulation of MAPK pathway (Beggs et al. 2015). Furthermore, the methylation of UGT1A1 in CRC was also found to affect the chemosensitivity to chemodrugs like irinotecan (Xie et al. 2014). Similarly, promoter hypermethylation of gene WNT5A (Wnt family member 5A) is found correlated to show efficient response to 5-FU in vitro (Jiang et al. 2017). Inconsistently, decreased promoter methylation of p16 was detected to show resistance to higher concentration of doxorubicin in CRC cell lines (Kubiliūtė et al. 2016).

The histone variants distributed along the chromosome influence the expression of genes. The histone modifications significantly regulate affinity of certain regions of DNA. These modifications were analyzed by techniques like single cell chromatin immunoprecipitation with DNA sequencing (scChIP-seq) (Rotem et al. 2015). Furthermore, there are various other techniques developed for sequencing such as single cell chromatin integration labelling followed by sequencing (scChIL-seq), single cell chromatin immune cleavage followed by sequencing (scChIC-seq), and single cell cleavage under targets and tagmentation (scCUT&Tag) (Ku et al. 2019). The genome-wide DNA hypomethylation was compared between the individual cancer cells and normal colon cells using single cell triple omics sequencing (scTrio-seq). This showed tumor lineages at the hypomethylated regions were enriched with long interspersed nuclear element 1 (LINE-1), long terminal repeats (LTRs), and heterochromatin regions/H3K9me3. Additionally, the tumor lineages with hypermethylated regions were also enriched with H3K4me3, CpG islands, and open chromatin. The DNA methylation degrees detected in CRC cells were highly correlated with densities of heterochromatin with histone modification H3K9me3 (Bian et al. 2018). The trimethylation of H3K9 plays a significant role in silencing the FAS transcriptional region in the cancer cell to escape from the immune control. However, a histone methyltransferase inhibitor verticillin A was identified to inhibit H3K9me expression in vitro at the promoter region of FAS in the CRC cells promoting the expression of FAS (Paschall et al. 2015). Thus, histone methylation could be a promising target for CRC therapy.

1.3 Applications

The revolution in biotechnology is potentiated by next-generation sequencing (NGS) that focuses on the study of transcriptome and genomic variations efficiently with high accuracy. The study was also potentiated by integrating NGS into various other projects including ENCODE, modENCODE, and Mouse ENCODE (Bernstein

et al. 2012; Yue et al. 2014; Roy et al. 2010). The application of NGS includes various sequencing systems like targeted NGS, whole exosome sequencing, whole genome sequencing, RNA-seq, and ChIP-seq (Wang et al. 2009; Bailey et al. 2016; Park 2009).

The CRC inter-patient heterogeneity can be analyzed via single biopsy that ensures the detection of genomic cancer genes and targetable mutations. The novel tumor-associated mutated driving genes both for familial and sporadic CRC responsible for proliferation, genome stability, apoptosis, RNA processing, and immune evasion were detected through genome and whole exome analysis (Huyghe et al. 2019; Bailey et al. 2018). This research on rare variants correlated with the types of CRC promises better understanding of the diseases at the gene level and potentiates to make future drug development (Huyghe et al. 2019; Chubb et al. 2016). For instance, about 70–75% of CRC cases are potentially found as single nucleotide variants, and copy number alterations include Wnt, PI3K, and Ras, highlighting the applicability for drug designing for targeting combinational drugs in patient centric trials (Bailey et al. 2018).

The ITH is discovered with variable degree in CRC. Previously, researchers determined that the whole exosome sequencing at multiple regions of primary and metastatic sites of tumor showed higher levels of ITH and sub-clonality determining the diversification. Significantly, these are found effective for aggressive therapies as well for early detection of the disease (Hu et al. 2019). As already discussed, ITH is the characteristic differences at genetic and molecular level between tumor cells within a single tumor (Punt et al. 2017). The study of CRC done via The Cancer Genome Atlas (TCGA) and Consensus Molecular Subtypes are the advanced types, however hindered due to incapable of capturing ITH for bulk profiling. Therefore, single cell sequencing is the available technology now to explore the tumor cells from the given population to investigate ITH and determine the genomic alterations. The tumor heterogeneity on the basis of environmental alterations associated with CRC progression is performed by combining the single cell DNA and RNA sequencing in a mouse model. About 200 cells in the mouse showed ITH that is altered due to the exposure of environmental changes. Subpopulation like cells showing mesenchymal-epithelial transformation (MET) were determined from the study that showed similar results when compared with TCGA (Ono et al. 2019). However, ITH has also been limited as the evolutionary principles in relation to designing the history of CRC by ITH are still unclear. Therefore, deeper investigation for cell-to-cell heterogeneity is highly essential to define the mechanisms responsible for tumor progression and metastasis. Chemoresistance is the most common obstacle for the CRC therapy that results in recurrence and metastasis. Targeted therapies are approved for mCRC; however, they are limited due to the heterogeneity. These therapies are found effective only for Ras wild-type patients and are ineffective for Ras mutations (Bartczak et al. 2017). Additionally, they are also found unresponsive toward CRC patients with mutations in BRAF and PI3KCA (Tamborero et al. 2018). Therefore, targeting the heterogenic sites and developing the drugs against them through advanced techniques are very much essential. In this regard, single cell transcriptomics plays crucial role in developing personalized medicine via developing associated prognostic markers. Another powerful tool is scTrio-seq that

is capable of identifying methylation, mutations, and transcriptome for a single cell (Bian et al. 2018).

CTCs shed into the circulating system separated from the primary tumor. These are rare metastatic cells that form secondary tumor at distant organ or tissue (Ferreira et al. 2016). Additionally, researchers and clinicians are now focusing on assessing the samples of non-invasive acquired plasma targeting for detection of biomarkers essential for diagnosis via NGS analysis for CTCs. These cells include circulating free DNA (cfDNA) and circulating tumor DNA (ctDNA) (Strickler et al. 2018; Rothwell et al. 2019; Zill et al. 2018; Cohen et al. 2018; Peeters et al. 2019; Kim et al. 2018). Similarly, the liquid biopsy for identifying the cancer evolution and predictive markers is done during the surveillance of patient. The use of CTC is now highly encouraged for better understanding of the tumor biology and can be a novel standpoint in the field of oncology (Lim et al. 2019). Previously, EpCAM-based immunoisolation was done for CTCs taking six mCRC patients. This was combined with whole transcriptome microarrays, representing 410 genes related to cell migration, adhesion, proliferation, apoptosis, and cell signaling. This suggests that CTC could be a promising approach as a prognostic and predictive biomarker in the clinical management for CRC therapy (Barbazan et al. 2012). Six genes including AGR2, EpCAM, CEACAM5, KRT18, FGFR3, and CLDN3 were identified from CTC lines that are responsible for CRC growth, progression, and metastasis (Onstenk et al. 2015; Mostert et al. 2015). The plasma of cfDNA can detect tumor-associated gene alterations that drive mutations and drug resistance. For instance, a large scale of 20,000 patients with late-stage CRC showed tNGS of plasma cfDNA. Among these cfDNA detected in almost 20% of total cohort showed clonal evolution and variant associated with chemodrug resistance in response to treatments targeting mutations (Zill et al. 2018). cfDNA is also found advantageous in diagnosing tumor at its early stages (stages I–II). The preliminary analysis of the circulating cell-free genome atlas (CCGA) is highly encouraged than other CancerSEEK test (Cohen et al. 2018) that aims to enroll about 15,000 members for a large scale of clinical trial to evaluate the diagnostic efficiency of whole genome bisulfite sequencing of cfDNA, while profiling of ctDNA mutated genes as the predictive biomarkers is already in the settings of early clinical trials. These trials include detecting the patient stratification after their treatment with combinational drugs compared with their individual circulating variability (Rothwell et al. 2019). This was a promising strategy to potentiate disease control and determine sensitivity for drugs especially in mCRC patient that aids for personalized cancer therapy.

1.4 Conclusion

CRC is now a public health problem worldwide due to its increased incidence and mortality rate. The genes responsible for CRC progression and metastasis are highly susceptible and respond variedly to the therapy. The heterogeneity nature of CRC for disease progression and tumor microenvironment can be studied significantly by the single cell technique analyzing single cell from the tumor. These techniques

enable diagnosis or prediction of disease at an early stage and promote targeted therapy. Additionally, the single cell techniques that include genomic and transcriptome analyses, ITH validation, and matching circulating tumor cells would promote individualized therapy with sensitive drug targeting. The heterogeneity between cell and cell would also promote multi-targeted treatments as per matching the mutational landscape of intra-patient. However, single cell techniques remain limited due to cost and time-consuming process. Additionally, this process includes unavoidable technical errors, sequencing bias, and abilities of experimenter. The development of multi-omics single cell techniques is now highly advanced and would conduct analysis of genome, proteome, epigenome, and transcriptome at single cell level. A timely monitoring of the patient for the diagnosis and therapy to prevent the disease can be realized easily through single cell technique. Thus, single cell technique would be an indispensable procedure in the medical field to treat CRC and explore the disease at the gene level more expediently.

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Chapter 2
Dietary Habits and Global Incidence of Colon Cancer

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Abstract Colorectal cancer (CRC) is one of the fast increasing cancers and currently stands third in the worldwide list. In the last decade, CRC was more common in Western countries than in Asia. However, currently, CRC incidence rate is high in Asian countries and accounts for the greatest number of cancer death. Therefore, different factors responsible for these changes in CRC incidence rate in Western countries and Asian countries need to be identified. We also need to determine the changes which led to the increased rate of cancer incidence in Asian countries. There are many possible reasons for such an increase in CRC incidence rates such as the adoption of Western dietary habits, lifestyles, extensive use of pesticides, and water pollution. Cancer is a preventable disease as various precautionary measures have been shown to reduce the risk of cancer initiation both in familial and sporadic cancers. If we identify the accurate etiology of CRC in a specific region, cancer incidence can be minimized by simply avoiding the chances of carcinogen exposure. In the case of CRC, these carcinogens are introduced in the human body from contaminated food and water, or come from food preservatives, or produced during food processing and cooking. Studies have shown that the use of alcohol and smoking also contribute to CRC initiation in many ways. These factors increase the likelihood of cancer progression in familial CRC but are solely responsible for sporadic CRC. Long-term avoidance of the bowel diseases which

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can be easily managed by the medicines and lifestyle changes in initial stages also contributes to the progression and cancer initiation. The difference in genetic background and extent of carcinogen exposure further influence the different sensitivities of colon cancer among individuals. The declining immune responses which are also influenced by many factors contribute to the development of CRC. This chapter focuses on identifying the factors which are responsible for different incidence rate in Western and Asian countries. This may help to pinpoint the accurate factor responsible for different incidence rates among countries.

Keywords Diet · Colorectal cancer · Western diet · Etiology · Molecular · Lifestyle factor · Environmental factors · Pesticides · Immune response · Microbiota

Abbreviations

AA	African American
ADH	Acetaldehyde dehydrogenase
ALDH	Aldehyde dehydrogenase
BaA	Benzo[a]anthracene
BaP	Benzo[a]pyrene
HCAs	Heterocyclic amines
MeD	Mediterranean diet
MPE	Molecular pathological epidemiology
NNN	N′-Nitrosonornicotine
NOCs	N-Nitroso compounds
OCPs	Organochlorine pesticides
OPPs	Organophosphorus pesticides
PAHs	Polyaromatic hydrocarbons
SCFAs	Short-chain fatty acids
t-t-DDE	Trans,trans-2,4-decadienal

2.1 Introduction

Colorectal cancer (CRC) ranks second in female and third in male among the most commonly occurring cancer worldwide. In the USA, CRC lies the fourth rank in terms of new cancer cases and third in the rate of death (GLOBCON 2018; Rawla et al. 2019). The high incidence rate of CRC is seen in developed countries, but over the years, it has been shifted to developing countries, mainly due to westernization, less screening program, or successful early detection tests in Western countries (Carroll et al. 2014). Risk factors for CRC predisposal can be grouped into dietary factors, non-dietary factors, and genetic defects. Other factors that influence the

incidence rate of CRC are age, greater body fat, awareness, economical status, and screening programs of the government to diagnose in early stages. Dietary factors consist of more than 30% of all CRC cases and are mostly induced by exposure of carcinogen from either food or water of regular human meal. Persistent exposure to carcinogens may result in the development of colonic malignancy throughout the decades. Diet-induced carcinogens are not only responsible for colorectal cancer but are also known to play an important role in other malignancies. The dietary habit varies among the countries so as the rate of CRC incidence. Historically, the highest incidence rate was noted in Western countries (Australia, USA, North America, Western Europe) and low in developing countries (India, the Middle East, and South America) (Center et al. 2009a; Torre et al. 2015). CRC is the fourth and second most diagnosed and cancer-related deaths in North America (American Cancer Society 2008). However, the trend is reversed in the last decade, mainly due to the acceptance of Western dietary habits, lifestyle, and extensive use of pesticides. The Western pattern adopted by developing country includes processed meat, saturated fats, fat products (butter, margarine, mayonnaise, fries, fast foods), refined grains, sweets, alcohol, smoking, and less physical activity (Center et al. 2009a; Center et al. 2009b) instead of an earlier dietary pattern of high-fiber plant-based food, vegetables, fruits, and low intake of fat which is eventually minimized (Popkin 1994; Siegel et al. 2011). The role of dietary pattern in CRC has driven attention after an observational study conducted in African populations showing low CRC incidence with high fiber intake (Burkitt 1971). A population-based study has found most of the cancers occurring in Australia are mainly due to inadequate intake of fruit and non-starchy vegetables, and out of all CRC cases, one in six is because of not including a sufficient amount of fiber in diet (Nagle et al. 2015). A multicase-control study of MCC-Spain has done the comparative study of the Western diet with the Mediterranean diet (MeD) and prudent diet which has found no association with prudent diet but has found a decrease in CRC risk with MeD which concluded that consumption of fruits, vegetables, olive oil, and nuts and avoiding red/processed meat, refined grains, sweets, drinks, and juices can reduce the risk of CRC (Castelló et al. 2019). A MeD such as high consumption of fruits, vegetables, and complex carbohydrates, low intake of meat and fish, a glass of red wine, and olive oil has influenced the less or no risk of CRC (Farinetti et al. 2017). The red meat such as beef, pork, and lamb is muscle meat which contains a high amount of myoglobin and is the most preferred food of the Western countries. Processing of meat by salting, curing, heating, and smoking increases its microbiological stability, taste, and flavors. However, processing of red meat results in the formation of different carcinogens such as polyaromatic hydrocarbons (PAHs), heterocyclic amines (HCAs), and N-nitroso compounds (NOCs) which are linked to increased CRC formation (Sugimura 2000). The International Agency for Research on Cancer (IARC) has classified processed meat as a carcinogen and red meat as a probable carcinogen to human (Kim et al. 2013a). The red meat carcinogenic compounds generally adduct DNA, and heme iron present in the myoglobin of red meat promotes alkylating NOC and forms reactive oxygen species (ROS) in the colorectal epithelium and causes different types of DNA damage (Cascella et al. 2018).

The clinical feature of CRC also varies greatly between the different countries. Many factors influence CRC in terms of etiology, incidence, age, and the difference in urban vs. rural occurrence. There is a high proportion of poorly differentiated advanced stage CRC in Egyptian compared to Western countries. One of the bases for these differences could be the genetic or epigenetic differences that have limited support from the literature (Abdel-Rahman et al. 2017). Another important basis of these differences is environmental risk factor which is known to alter the gene expression and overall cell metabolism. However, there is a difference in carcinogen exposure, and the limit of these environmental factors can influence the incidence rate and clinical outcome. Together these diet-induced carcinogens account for the differences in the overall CRC incidence rate in rural and urban populations within any country and between the Western and Asian countries. Luckily the most desirable prevention modalities to CRC are also based on diet and involve avoidance of diet-induced carcinogen, use of chemopreventive agents to minimize the effect of carcinogen, and dietary fiber intake which reduces the time of exposure of carcinogen to the intestinal epithelium. Healthy lifestyle and habits are other important factors that reduce cancer initiation and progression by a different mechanism. Therefore, identification of dietary carcinogens by comparing the dietary habits among countries and their novel mechanism of carcinogenesis is urgently needed so that preventive modalities can be applied to reduce the CRC incidence rate. Identification of these environmental carcinogens and their route of entry can be used to plan effective preventive modalities such as avoidance of exposure or use of dietary chemopreventive agents. These preventive steps can reduce the incidence of CRC in affected areas and maybe the reason for the observed difference among the countries. In the present chapter, we will discuss the different potential CRC causing dietary carcinogen exposed by the population and pinpoint the region for different CRC incidence rate among countries. The molecular and clinical differences in the CRC etiology will also be discussed. Finally based on our understanding of CRC etiology, prospect of preventive strategies to minimize the CRC incidence rate is outlined. Figure 2.1 summarizes the different worldwide dietary habits and CRC incidence rate.

2.2 The Difference in Dietary Habits

There is a difference in the food habits of the Western and Asian countries, which influence the incidence rate of CRC. These differences are also shown to be strongly associated with lifestyle and difference in response (genetic and immune). Dietary habits play a vital role in detoxifying ROS from the body, binding with carcinogenic compounds, and making gut flora healthy by increasing good bacteria and decreasing the influence of pathogenic bacteria. There is also variation among different countries in consuming food. The following are the major dietary habits. Table 2.1 summarizes the different dietary habits and their relation with CRC.

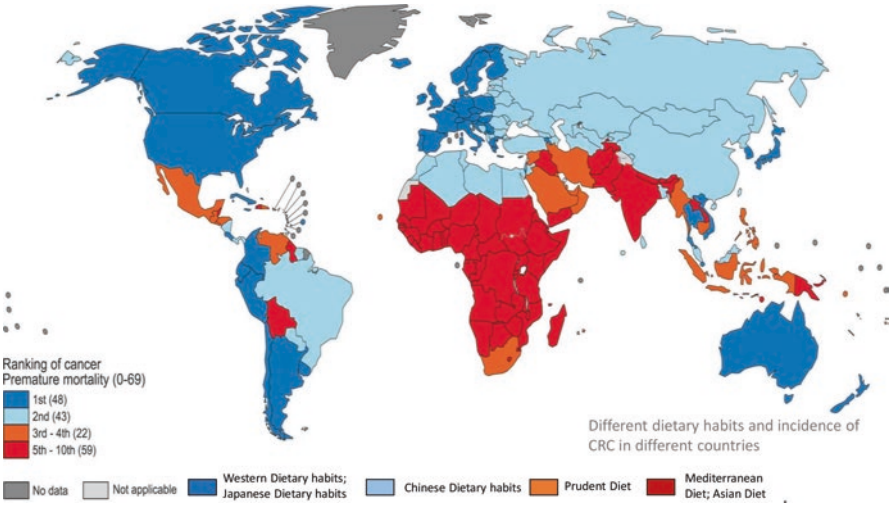

Fig. 2.1 The world map depicting the countries with high incidence of CRC and their different dietary habits. (Source: World Health Organization (WHO), 2018)

Table 2.1 Cohort studies showing association of dietary habits with CRC

Cohort study	Dietary pattern	Significance	Author, year
Argentina	"Southern Cone," sugary-diet, prudent diet	High sweet intake is related to the risk of CRC	Pou et al. (2012)
African American	Western diet – salted pork, turkey, beef smoked, pigtails	Affects women and increases the risk of CRC	Bovell-Benjamin et al. (2009), 2010)
MCC-Spain	Mediterranean diet – vegetables, fruits, olive oil, and red wine	Decreases the risk of CRC	Farinetti et al. (2017)
Nigeria	African diet – *cassava* in vegetable soup, including spices	Low incidence of CRC	Irabor (2011))
Shanghai	China – cholesterol-rich fish, eel, shrimp, and shellfish	Increases the risk of CRC	Lee et al. (2009)
Japan	Japanese diet – green tea, miso soup (*wakame*)	Previously, low incidence of CRC	Tokudome et al. (2000), Fujiki et al. (2018)
Japan case-control study	Beefsteak, pork steak, ham, yakibuta	Mostly men have a high risk of CRC due to intake of red meat	Wada et al. (2017)

2.2.1 The Western Diet

The Western dietary habits are generally adopted by developed country like the USA and have a higher intake of meat, processed food, potatoes, refined carbohydrates, high-fat dairy, eggs, butter, fries, sweets, soda, and snacks and a lower intake of vegetables, fibers, and whole wheat grain, which is associated with increased CRC risk (Fung et al. 2003; Fabiani et al. 2016; Moss and Nalankilli 2017). From the past few years, many countries have inclined toward the Western diet, which lead to an increase in various chronic diseases (Cordain et al. 2005) and cancer incidence concluded from various epidemiological studies (Moss and Nalankilli 2017; Key et al. 2002). One study has shown the high overall odd ratio of in taking Western diet and an increase in the risk of CRC when compared to individuals including fruits, vegetables, and low intake of red/processed meat (Tayyem et al. 2017). The red/processed meat is pro-inflammatory and produces ROS which is known to be associated with carcinogenesis (Cross et al. 2007). The study from various countries has shown different dietary habits in population and their risk to CRC. The cohort study from Argentina has shown three major dietary patterns which mainly included "Southern Cone pattern," sugary-diet pattern, and prudent pattern, which are characterized by (1) intake of red and processed meats, starchy vegetables, wine, fats, and oil; (2) a high sugar intake of drinks, sugar-added foods, beverages, fats, oil, fish, and poultry; and (3) high intake of dairy foods, fruits, vegetables, poultry, and fish, respectively, of which high-sugar diet has shown a high risk of CRC than the Southern Cone followed by prudent pattern, where prudent is less associated with CRC compared to the rest two diets (Pou et al. 2012).

From the survey, it's seen that African-American (AA) diet includes less intake of fruits and vegetables than White Americans (Kant and Graubard 2007; Fulgoni 3rd et al. 2007) which makes them highly susceptible to CRC, and it is advisable to switch to prudent diet (healthy diet including a good amount of vegetables and fruits) for the reduction in CRC rate (Makambi et al. 2011). The traditional food of AA mainly includes large amounts of salts, including salted pork fat and lard mainly consumed in the Black Belt region (Smith et al. 2006). The AA diet is considered as diet high in fat, high-sodium content, and frying which is related to high risk of cancer (Bovell-Benjamin et al. 2009). Some study has also shown the amount of red meat taken in the form of Whopper, Big Mac, and McDonald's consumed per week is higher in men. In AA, meat is mainly taken in the form of salted pork (roast, ribs, chops, pigtails, neck bones), chicken, beef, turkey, ham, sausage (bologna, beef smoked), and tuna; also carbohydrate-containing food (potato, pasta, macaroni, rigatoni cheese, noodle, pizza; sweets and low fruit and non-starchy vegetables) (Bovell-Benjamin et al. 2010). AA brings a large portion of fat in their diet mainly by fried poultry, processed luncheon meat, and bacon (Popkin 2007).

2.2.2 Mediterranean Diet (MeD)

MeD is considered a healthy diet to reduce the risk of CRC. It is common in countries near the Mediterranean Sea. It is also recognized as the "intangible cultural heritage of Italy, Greece, Spain, and Morocco" by UNESCO in 2010 (Trichopoulou et al. 2009). Mediterranean diet includes lots of fruits, vegetables, whole grain, fish, seafood, limited processed food (red meat), red wine, and olive oil (Schwingshackl and Hoffmann 2015). MeD diet decreases inflammation, maintains healthy weight (Boeing et al. 2012), and reduces carcinogens in the GI tract, and also the limited intake of red meat nullifies the risk. The benefits of red wine (alcohol) are limited depending on moderate consumption (Lachenmeier and Rehm 2012; Lee et al. 2007). Some study has shown the inverse relation of MeD and colorectal cancer (Farinetti et al. 2017; Schwingshackl and Hoffmann 2015). The MeD seems to give promising results, yet its definition differs in the region.

2.2.3 African Diet

The higher incidence rate of CRC is seen in Oceania (Australia) and Europe with 30 or more than per lakh range while in Africa and Asia 5 or less than per lakh lower incidence of CRC (World Cancer Research Fund International) (Haggar and Boushey 2009). There is a decrease in colorectal cancer incidence in the African population compared to North America (Butler et al. 2003) or other Western countries mainly due to dietary habits, and this is highlighted after Burkitt's epidemiological study. The West African diet mainly includes carbohydrate-based meal consumed with a soup containing vegetables while in rural and poor communities, meat is rarely consumed and fleshy fruits are taken in regular meal, *cassava* (consists of chemical tamarin, which is toxic to cancer cells) (Marandola et al. 2004) and also favor's variety of maize. The typical Nigerian food is hot and spicy including tomatoes, red chili, peppers, and onions cooked with vegetable or palm oil, containing the phytonutrients, which protect against cancer. In addition, Garlic and onions are widely used by Nigerian in cooking, and also curcumin (but not more than Indian cooking) and red pepper (capsaicin) which have anticancer property against colon cancer cells (Irabor 2011). Therefore, this may be the reason for the low incidence of CRC in this region.

2.2.4 Asian Diet

The burden of CRC in developed countries (Czech Republic, North America, Europe, Japan, Australia, and New Zealand) accounts for 59%, while in developing countries (Asia, Africa, Central, and South America), it accounts for only 41% in

spite of having large population sizes (Pathy et al. 2012; Bishehsari et al. 2014); these differences are mainly due to low consumption of sugars, calories, and fat-rich food and high intake of vegetables and fruits and avoidance of obesity and overweight by involving physical activity (Pathy et al. 2012). In India, the CRC incidence rate remained stable as in sub-Saharan Africa in the past two to three decades in comparison with an increasing trend in East Asian countries (China, Singapore, Japan, Malaysia, Korea, and Turkey) (Toyoda et al. 2009), where the 5-year prevalence rate of CRC is higher (IARC 2018). The incidence rate remained low in India for a long time probably due to traditional lifestyle patterns including consumption of more vegetables, fruits, and whole grains, low meat intake, and ingestion of Indian spices (curcumin, cumin, chilies, Amrita Bindu, which has variously shown anticancer activity) (Sinha et al. 2003) and also due to optimal physical activity (Nayak et al. 2009; Sullivan et al. 2011).

2.2.4.1 Chinese Diet

The incidence rate of CRC is increasing in low-incidence countries like China (Ji et al. 1998). It has been increased from 14 to 22 per hundred thousand in men and 12 to 19 per hundred thousand in women (Ji et al. 1998). In China, more than 2 lakh new cases and more than lakh deaths occurred due to CRC in 2012 (WHO); also the guidelines were made to control CRC incidence and mortality due to low vegetable and fruit intake, high red and processed meat, physical inactivity, alcohol drinking, and tobacco smoking (Gu et al. 2018). Back, in the 1970s, there is an increase in the consumption of animal foods in China (Yu et al. 1991), mainly in Shanghai, where there is also an increase in the incidence of CRC. The Shanghai Women's Health-based study has found cholesterol-rich fish, eel, and shrimp and shellfish consumption in their dietary intake and also the use of smoking as a cooking method, which is related to a higher risk of colon cancer (Lee et al. 2009). Previously, Shanghai-based study has also found an increase in the consumption of fat, poultry, and pork, smoked or salted fish, salt-preserved vegetables, and cured and processed meat, low intake of plant foods in their diet, and also less physical activity and increase in the prevalence of obesity (Chiu et al. 2003). Another China-based study of Shandong, reveals their dietary habit with a high-fat, processed food cooked in high temperature by using methods such as barbecue and frying. Also including some popular preserved foods like Brawn and kipper. In addition, they also include adequate amount of garlic in diet, whose anticancer activity is still controversial (Wang et al. 2018).

2.2.4.2 Japanese Diet

Previously, there is a limited incidence of colon cancers in Japan (Tokudome et al. 2000), mainly due to the limited intake of calories of the traditional Japanese diet, but from the last 40 years, it has been increased in both genders due to the adoption of Western dietary pattern (Minami et al. 2006). The traditional Japanese diet

includes vegetables, fiber, miso soup having vegetable *wakame* (Hirayama 1982) (containing fucoxanthin), green tea (rich in antioxidants), and soy products (isoflavones and saponins) (Fujiki et al. 2018). Changing this dietary pattern may be the reason for the increased incidence of cancer in Japan. In Japan, the follow-up study from Takayama has found that intake of red meat or total meat (poultry, fish) is more in men than women; here the food item intake for red meat products including beef steak, pork steak, pork cutlet, grilled meat, grilled offal, and liver and processed meat such as ham, sausage, bacon, and *yakibuta* (Chinese-style roasted pork) showed a hazard ratio of 1.36 (total meat) and 1.44 (red meat) (Wada et al. 2017).

2.3 Fruits and Vegetables (F&V)

F&V contains a high amount of micronutrient, provide tastes, varieties, low calories, and contain protective properties. The low intake of F&V contributes to approximately 2.7 million annual deaths and approximately 19% of gastrointestinal cancer (Ezzati et al. 2004; New 2003). Low consumption of F&V also acts as a sixth main risk factor for mortality in the world (Ezzati et al. 2004). The majority of developed countries like the USA, Australia, Europe, and the UK consume less than the daily recommend F&V. Also, in some of the developing countries including India, 77.6% of men and 78.4% of women consume fewer F&V than recommended (Hall et al. 2009). MeD consuming population has shown a low risk of CRC, and this is attributed mainly due to the high content of vegetables, fruits, and a variety of herbs and spices (like parsley, saffron, thyme, basil, rosemary, oregano, and sage) (Kontou et al. 2013). It is recommended to include non-starchy vegetables (like broccoli, carrots, celery, peppers, tomatoes, Zucchini) and some of the low-salt-containing vegetables (cruciferous cauliflower, cabbage) which have sulfur-containing glucosinolate, which metabolizes to isothiocyanates (ITCs) and indole-3-carbinol (I3C) by colonic bacteria and shows anticancer properties (Higdon et al. 2007). In addition, tomatoes and onions have taken as a salad or as a part of vegetables in a diet shown to contain apoptotic activity in a mice (Kim et al. 2015), model due to the presence of an active compound glycoalkaloid α-tomatine (He et al. 2014). (Kim et al. 2015), onion flavonoids (He et al. 2014). In support to this, an EPIC-Italy-based study has advised taking "Olive oil & Salad dietary pattern" which includes raw and leafy vegetables, and tomatoes associated with the MeD, and low risk of CRC (Sieri et al. 2015; Masala et al. 2007).

2.3.1 Dietary Fiber

From many case-control cohort studies, the inverse association between dietary fiber, whole grain intake, and risk of CRC has been concluded (O'Keefe 2019) which highlights the beneficial underlying properties of dietary fiber in preventing CRC by increasing the consistency of fecal matter, decreasing transit time, and

restricting exposure time of carcinogen to the colonic epithelium; it also binds to the secondary by-products of bile juice. Moreover, it increases the beneficial bacteria in the colon, thereby enhancing the microbiome diversity and decreasing the exposure of colon to deleterious compounds. The microbiomes in the colon environment ferment the dietary fibers and produce short-chain fatty acids (SCFAs) which influence the immune response and signaling pathways and have shown its anticarcinogenic properties in various studies (Simpson and Campbell 2015).

2.4 Grains

With an increase in urbanization, the use of whole grains and grain products which consist of bran, germ, and endosperm is reduced, and that of processed grains such as white rice, bread, or pasta is increased. A variable amount of fibers, vitamins, and other micronutrients are present mostly in the germs and the outer layer of the grains. These fibers and micronutrients present on the germ and outer layers are removed during the refining and processing. Studies have shown the risk of CRC can be lowered through a high intake of the whole grains (Thielecke and Nugent 2018). An epidemiological study has considered alkylresorcinols and phenolic lipids as a circulating biomarker for determining its presence in diet, as it is mainly found in bran and rye. The presence of alkylresorcinol in plasma is associated with reduced risk of distal colon cancer but not with overall colorectal cancer as seen in the population of France, Germany, Italy, the Netherlands, Spain, Denmark, and the UK (Kyrø et al. 2014; Ross 2012). Hence, this could suggest a higher intake of whole grain in a diet which is also a source of high-quality carbohydrate, having a low glycemic index and slow absorption, also beneficial in controlling insulin which plays an important role in colon carcinogenesis (Eleazu 2016).

2.5 Meat

Meat is rich in protein and saturated fatty acid (SFAs), usually consumed in Western countries. Its association with CRC risk is controversial in terms of risk, location, and gender. A Korean-based study has shown meat intake causing cancer in the proximal colon of men and rectal in women (Shin et al. 2011). The meat intake each of 100 g/day increases the risk of CRC by 12% (Vieira et al. 2017). From the past few decades, there is an increase in the consumption of pork, eggs, and milk products in China to 100%. A USA-based meta-analysis has found a positive association between red meat and CRC risk for every 100 g per day consumption of red meat and 50 g for processed meat (Chan et al. 2011). Red meat includes beef, veal, pork, lamb, and also poultry (chicken, fish) (Humans IWGotEoCRt 2018). The type of carcinogens produced from cooking meats depends on the type of meat, method used to cook it, temperature, and time taken for cooking (Knize et al. 1994).

The exposure to carcinogens from processed meat-producing NOC is exogenous, whereas endogenous exposure depends on the amount of intake (Cross and Sinha 2004). The different cooking methods include pan-frying, grilling, barbecuing, and smoking which produce a high level of mutagens responsible for carcinogenesis (Joshi et al. 2015), and their mutagenic activity is detected in the urine sample of humans (Peters et al. 2004). The AA contains a high amount of protein and carbohydrates and high amount of grits in breakfast; although it is not harmful, but the method of its preparation by high addition of fat and salt makes a big concern (Patterson et al. 1995). Generally, fast food is prepared with saturated fats and salt and also involves frying. In a 3-day study of dietary intake of food in the AA population, 27% female and 31% male recorded with high intake of fried and pan-fried red meat. The frying of foods gives a golden brown texture, which enhances the flavor and taste of food, and it is the main cooking method liked by AA (Bovell-Benjamin et al. 2009; Dirks and Duran 2001). Many mechanisms explain the carcinogenicity of red meat; one of these is the presence of heme iron in red meat that enhances the risk of CRC, as free iron participates in Fenton reaction and produces ROS and causes lipid peroxidation. These by-products could lead to cellular damage, although there is a limitation in epidemiological evidences (Cascella et al. 2018) to support the hypothesis. Hence, consuming meat (high-caloric diet) can increase the risk of CRC; however, substituting it with other sources of digestible proteins such as legumes, whole grain wheat, fruit, and white meat such as fish and poultry might reduce the risk of CRC.

2.6 Carcinogens Produced by Cooking Methods

A different method of cooking is applied by different populations of the world, depending upon the ingredients, temperature, and time taken for cooking; these cooking methods produce many carcinogenic compounds like NOCs, HCAs, and PAHs which can cause DNA damage (Cascella et al. 2018). From the population-based study, some of the cooking methods and carcinogens produced from it are concluded. Few common cooking methods are discussed below.

2.6.1 Barbecue (BBQ)/Grilling

BBQ or grilling is a common cooking practice used worldwide for eating meat (beef, pork, chicken) at high temperature in burning charcoal which produces HCAs and PAHs detected in fumes and fine particles ranging from 0.18 to 1.8 micrometer (Lao et al. 2018; Lee et al. 2016). The release of these harmful compounds depends upon the precursors in meat including creatinine, amino acids, and carbohydrates present in it. Among the four PAHs (benzo[a]anthracene (BaA), chrysene (Chr), benzo-B-fluoranthene (B[B]F), and benzo[a]pyrene (BaP)), BaP is the most studied

and used as a marker for carcinogenic PAHs in food (Lee et al. 2016; Kazerouni et al. 2001).The grilled or barbecued meat contains PAHs because of exposure of smoke to pyrolysis of fatty juices, which may be the reason for an increase in the risk of colon cancer. A Danish-based study has also concluded that the darker the skin color of meat, the higher the concentration of HCA, which has also found the highest concentration of HCA (9H-pyrido[3,4-b]indole (norharman) and 2-methyl-β-carboline (harman) in beef than pork and chicken (Aaslyng et al. 2013). In the USA, high-temperature or open-flame cooking for red meats including broiling and barbequing increases the risk of type II diabetes among meat eaters (Liu et al. 2017). The difference in the dietary habits of AA has influenced the CRC rate, where the high CRC incidence is found in AA mainly due to exposure of mutagens produced from cooking meat at high temperature and external charring, mainly achieved by barbeque which produces HCAs (Butler et al. 2003), PhIP, 2-amino-3,8-dimethylimidazo[4, 5-f]quinoxaline (MeIQx), and BaP (Butler et al. 2003). In Nigeria, due to insufficient supply of electricity, meat is not refrigerated but is deep-fried for preservation. The charcoal roast known as "suya" in Nigeria (Irabor 2014) is another method for cooking meat.

2.6.2 Frying

Frying is a common method used in almost every cooking practice. Carcinogens produced from frying depend on the type of frying method and oil used. The cooking fumes from frying contain ultrafine particles that affect the lungs and also have mutagenic activity (Zhang et al. 2010). It is classified as a "probable carcinogen to humans" (Group 2A) by IARC (2010a). The fumes mainly contain various aldehydes such as trans,trans-2,4-decadienal (t-t-DDE), trans,trans-2,4-nonadienal, trans-2-decenal, trans-2-undecenal, and naphthalene (Sjaastad et al. 2010). A study by Peng et al. (2017)) considered three cooking methods used in frying (stir-, pan-, and deep-frying), and the highest aldehyde emission is found in deep-frying than the pan- and stir-frying. This study also suggested the use of stir-frying and palm or rapeseed oil, so that there will be less production of t-t-DDE. A study on mice has found that continuous 4 weeks or more of fried meat consumption can cause DNA damage in colon cells (Sanz-Serrano et al. 2020).

2.6.3 Roasting

Coffee is taken as a drink in most of the countries. The processing of coffee involves roasting for the enhancement of color and flavor. But the presence of PAHs in coffee has made a concern, among which phenanthrene, fluoranthene, and pyrene are found in higher amounts with concentration up to 0.79μg/L, although coffee does

not suggestively contribute to the daily human intake of carcinogenic PAHs (Orecchio et al. 2009; Zelinkova and Wenzl 2015).

2.7 Carcinogen from Food Processing

Many believable mechanisms support carcinogenesis from red and processed meat and CRC risk (Cross et al. 2007). For example, cooking meat at high temperature releases NOCs which cause methylation of nucleotides (Joosen et al. 2009), and HCAs including the highly mutagenic 2-amino-1-methyl-6-phenylimidazo (4,5-b) pyridine (PhIP) induce the formation of bulky DNA adducts (Jamin et al. 2013). Studies from rodents and humans have shown the role of NOC and HCA in KRAS and APC mutations and are linked to CRC initiation (Fahrer and Kaina 2017). Another highly methylated DNA adduct O6-methylguanine (O6-MeG) arrises after exposure to alkylating carcinogenic compounds such as through smoking. These alkylated DNA adducts get repaired by base excision repair (BER) and by O-6-methylguanine-DNA methyltransferase (MGMT) (Wirtz et al. 2010). MGMT removes the methyl group from the O6-MeG lesion and restores the guanine base and itself degraded by ubiquitin/proteasome pathway (Fan et al. 2013). If this repair does not get fixed by MGMT, the O6-MeG adducts will mismatch with thymine and lead to transition mutation and eventually chromosomal instability. MGMT has been found hypermethylated on the promoter region of human colorectal cancer, hence decreasing its expression (Halford et al. 2005). Besides, it also repairs O6-(4-oxo-4-(3-pyridyl)butyl)guanine engendered by tobacco (Mijal et al. 2004). Hence, the expression of MGMT can prevent DNA damage and minimize cancer risk; this is also found regulated by natural compounds like cysteine prodrugs and curcumin which elevates its level in colon cancer cell lines and in various in-vivo studies (Niture et al. 2007). Therefore, this can be concluded that MGMT protects normal cell to get transformed against NOC-induced DNA damage and CRC initiation, but the daily intake of the diet containing NOC such as processed meat could reduce MGMT activity in GI and increase the O6-MeG lesions leading to increased risk of CRC.

2.8 Carcinogen from Food Preservation

Preservatives are added in food to prevent food spoilage from bacteria, fungus, and yeast so that shelf life and taste of food can be maintained for a longer time. The common preservative used for preserving food in packed food is salt and in Chinese food monosodium glutamate (MSG).

2.8.1 Salt

Salt is a commonly used preservative in preserving food, maintaining texture, flavoring, fermentation (Johanningsmeier et al. 2007), and preventing microorganisms' growth. Over 75% of salt in the food of Western countries come from processed or packed food (James et al. 1987), i.e., in meat products (ham, bacon, hot dogs), in fish sauce, in soy sauce, in dairy products (cheese, butter, condensed and evaporated milk, frozen desserts), and in baking products (bread cookies, cakes, pastry, pizza, breakfast cereals), while in developing countries, it mainly comes in diet from high salt added while cooking (Ravi et al. 2016). WHO recommends less than 5 g (~2 g sodium) of sodium per person per day to prevent chronic diseases; however, the intake of salt has increased in many countries like the USA (~9.1 g NaCl/day), and high consumption of salt is found in Asian countries (e.g., China, having the highest consumption from the past 5 years, and India [~11 g NaCl/day]) (Johnson et al. 2019). Some epidemiological studies have shown the relation between salt intake and the risk of CRC (Tsugane 2005; Murata et al. 2010), whereas some has not found any risk of CRC. It has been proposed that foods preserved by salting increase the risk of gastric cancer by the World Cancer Research Fund (WCRF 2016; Glade 1999; Diet 2016), considering mainly pickled vegetables or salted fish (Diet 2016). The daily intake of salt has been far extended in Korea (127.72 g/day) in taking their traditional dish *kimchi* (salted and fermented vegetable) than Japanese pickle (10.96 g/day) (Kim et al. 2016a). A Korean meta-analysis has proved its association with an elevated risk of gastric cancer (Yoo et al. 2020). The dietary salt damages epithelial cells and increases the risk of gastric cancer in patients with *Helicobacter pylori* infection (Thapa et al. 2019). The exact mechanism is still unknown, yet some animal studies have shown NaCl toxicity increases the S-phase cells (Charnley and Tannenbaum 1985) and its excessive concentration leads to carcinogenesis or tumor development (Omberg et al. 2009). So, management of salt intake is necessary and can be done by using salt substitutes such as herbs, spices, and lemon juice, and instead of ready-to-make instant food, self-cooking could be best.

2.8.2 Monosodium Glutamate (MSG)

Another preservative that is widely taken in food is MSG, which is used as a flavor enhancer in various processed foods (processed meat, canned vegetables, soups, sauces, dried bouillon cubes, and salty flavored snacks). Previously, umami is considered as a specific taste of MSG in Asia and then in Western countries (Zanfirescu et al. 2019). There is an increase in the consumption of MSG in Western countries with approximately 1.0 g daily intake (Beyreuther et al. 2007). The effect of MSG is controversial where some agencies claim their consumption to be safe, suggesting it to be a precursor amino acid (glutamate) for glutathione formation and an

excitatory neurotransmitter in CNS (Meldrum 2000). In contrast, its higher expression could damage the brain. In an animal study, it has shown inducing colorectal carcinogenesis by influencing diabetic metabolic conditions (Hata et al. 2012; Peeters et al. 2015). MSG is also referred to as "China salt" in many countries; from a long time, it has been linked to obesity, metabolic disorders, and Chinese restaurant syndrome (He et al. 2011; Niaz et al. 2018). In addition, Brawn and kipper are types of preserved foods taken by Shandong people in China.

2.9 Carcinogen from Pesticide Uses

Pesticides are used in agriculture for preventing crops from damage by biological agents, but it is an unavoidable fact that it can contaminate various food resources such as fruits, vegetables, fish, poultry, and meat (Nagao and Sugimura 1993) (Table 2.2). The increasing use of pesticides in various countries in the world makes a big concern. An Italian-based study has found 15 genotoxic pesticides in Italian

Table 2.2 Summary of pesticide uses and alteration of signaling pathways contributing to CRC

Pesticides	Chemical name	Country	Food	Pathways affected
Organophosphorus pesticides (OPPs)				
Chlorpyrifos	[O,O-Diethyl-O-(3,5,5-trichloro-2-pyridyl)-phosphorothioate]	USA, India (Narenderan et al. 2019), China (Xu et al. 2018)	Carrot, potato, celery, pak choi, leeks	EGFR/ERK1/2 (Suriyo et al. 2015)
Glyphosate		USA (Myers et al. 2016)	Soybean, maize (Swanson et al. 2014)	
Organochlorine pesticides (OCPs)				
DDT-(DDE)	p,p'-Dichlorodiphenyldichloroethylene	Egypt (Soliman et al. 1997) China (Chen et al. 2004)	Rice	Wnt/β-catenin and Hedgehog/Gli signaling (Song et al. 2014b)
Endosulfan (insecticide)		Mexico, South Africa, India (Dewan et al. 2004)	Vegetables, fruits, nuts, fish (Canlet et al. 2013), wheat flour	IL-6, TNF-α level increase and increased expression of β-catenin, P-selectin (Téllez-Bañuelos et al. 2016)
β-HCH, γ-HCH	Hexachlorocyclohexane	Brazil (Martin et al. 2018) Africa (Olisah et al. 2020)	Food, water, animal tissue (Teófilo Pignati et al. 2018)	Low expression of AChE, p16, and MGMT hypermethylation (Abolhassani et al. 2019)

foods (Dolara et al. 1993). Serum analysis on different populations of human samples from 35 countries has suggested that pesticides increase mortality rates related to various cancers including colon cancer (Wang et al. 1988). In the USA, the increased use of organophosphorus pesticides (OPPs) derived glyphosate (trade name "Roundup") in soybeans and maize (Swanson et al. 2014) is a big concern, as it is a health hazard and causes autism, kidney failure, and Alzheimer's disease and drives mutations that can lead to cancer. It is also categorized as "probable carcinogen to humans" by WHO (Myers et al. 2016). The neonicotinoid insecticide is used worldwide and has been found to increase in soybeans (34–44%) and maize (above 79%) in the USA (Douglas and Tooker 2015); also a significant level of pesticide is found in soy products (Nyman et al. 2003), although some studies does not found it significant. In several countries, pesticide use has been increased from the past decades, such as in Brazil where agriculture is the main income source, which accounts for maximum CRC incidence and mortality in men and women from the past 14 years (Uyemura et al. 2017). Brazil is the biggest exporter of pesticides (Rocha and Grisolia 2019), and its increased exposure in Brazil plays a major role in colon malignancy (Martin et al. 2018). The study done on animals residing in the Xingu River basin of Brazil has found a higher concentration of organochlorine pesticide (p,p′-DDT, endosulfan, and HCH) contamination in their tissue, specifically in turtle which is the main source of protein for them (Teófilo Pignati et al. 2018).

Pesticides such as organochlorine pesticides (OCPs) and their derivative dichlorodiphenyltrichloroethane (DDT) and their major metabolite p,p′-dichlorodiphenyldichloroethylene (DDE) are mainly responsible for proliferation in colorectal carcinoma by activating Wnt/β-catenin signaling pathway and inducing oxidative stress (Song et al. 2014a). Figure 2.2 summarizes the pesticide-induced

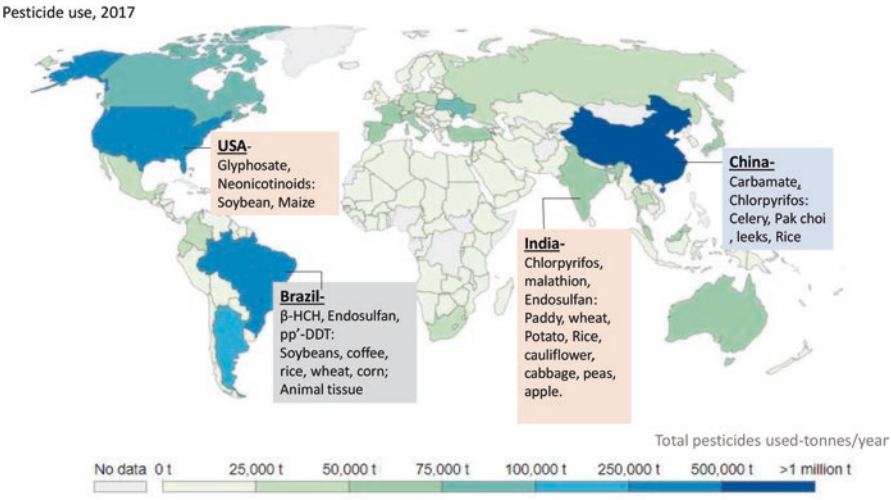

Fig. 2.2 Summary of pesticide uses in different countries. (Source: UN Food and Agricultural Organization (FAO) 2017)

alteration in signaling pathways. Past then, OCPs are also found higher in the serum of Egypt CRC patients (Soliman et al. 1997). Although these pesticides are banned in many countries, they are extensively used in the African continent. A study by Olisah et al. (2020)) has reviewed that hexachlorocyclohexane (α-HCH, β-HCH), DDTs, and endosulfans are the most prominent pesticides found in the environment, food, water, and soil which can increase the chances of coming in the food chain in the African population. Also, DDT's higher concentration found in breast milk has made great concern (Olisah et al. 2020). Endosulfan is considered a moderately hazardous pesticide (class II) by WHO (2019; 2020). A Korean-based hospital study has concluded that regular exposure of persistent organic pollutants (POPs) like OCPs and polychlorinated biphenyls (PCBs) can increase the risk of colorectal polyp formation and its deposition in adipose tissue (Lee et al. 2018). In India, being an agrarian country, high cultivation of crops, vegetables, and fruits occurs, and to protect them from insects, pests, and fungus, pesticides are widely used. The common pesticides used are organophosphorus (methyl parathion, chlorpyrifos, malathion) in various season-grown vegetables (Bhanti and Taneja 2007). A western Indian Himalayan region study has found higher contamination of OPs (methyl parathion and triazophos) in fruits (apple, grapes) and vegetables (cauliflower, cabbage, peas, potato), which is also related with health risk in children (Kumari and John 2019). Chlorpyrifos (84.90%) and quinalphos (71.69%) are highly found in a sample taken from fruits and vegetables cultivated in Nilgiris, South India; mostly carrot and potato are contaminated with pesticides (Narenderan et al. 2019), and these OCPs and OPs may induce the risk of CRC in humans (Abolhassani et al. 2019). Also, in some of the pesticides used in China (OPs, carbamate, pyrethroid,

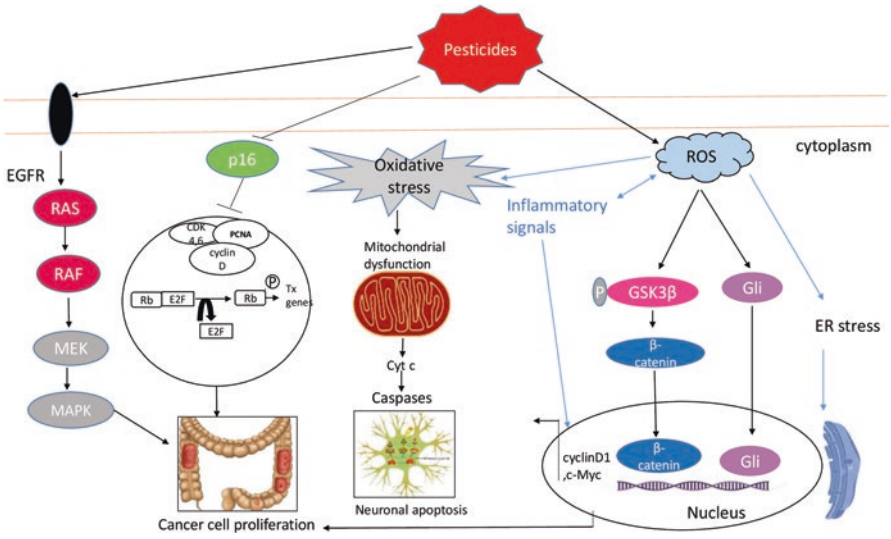

Fig. 2.3 Alteration of signaling pathway leading to CRC by pesticides

and triazine), chlorpyrifos is found in higher concentrations in celery, pak choi, and leeks (Xu et al. 2018). Figure 2.3 summarizes the different types of pesticide uses in different countries.

2.10 Environment Pollutants

In Asia, more rectal cancers are found mainly due to environmental factors, such as from water pollution, pesticides, or fertilizers used. The incidence rate of rectal cancer is 50% more in Asia compared to Europe and North America (Deng 2017). Mainly, solid cancers drive mutation with an increase in age, due to late diagnosis at the age of 50 years or more (de Magalhães 2013). The current data indicates that the CRC incidence is increasing in younger age groups or below 50 years of age (Siegel et al. 2014), which may be an indication in contribution of environmental factors in our food, drinking water, or pesticides coming vigrously through our food chain.

2.10.1 Aflatoxins (AFB1)

AFB1 are produced from molds growing on crops, contaminating harvests, and are naturally occurring carcinogens. Countries with a hot and damp climate such as Southeast Asia and China favor the growth of aflatoxin-producing fungi that increase the change of exposure of the food-born carcinogen (Sun et al. 2017). Aflatoxin is characterized as a carcinogen category (Group 1) and potentially carcinogenic to human category (Group 2B) by IARC (International Agency for Research on Cancer 2002). Its exposure to humans can be from the ingestion of contaminated food, beverages, and drinking water and also by inhalation of aerosol particles in the environment (Novak Babič et al. 2017; Kumar et al. 2017). The AFB1 carcinogenesis study on hepatocellular carcinoma (HCC) has shown that carcinogenesis occurs through DNA adduct formation and lipid peroxidation generating acetaldehyde and crotonaldehyde which induce DNA damage at codon 249 of p53 and also impair DNA repair system (Weng et al. 2017). AFB1 bioactivation occurs in liver where it gets converted to 8,9-epoxide (AFBO) by enzyme cytochrome P450 (CYP450) and has capability to form adducts by binding to DNA, RNA and proteins. The contamination of AFB1, AFB2, AFG1, and AFG2 was recommended to be less than 20 ppb in the USA while in the UK and Japan 4 ppb and 10 ppb, respectively (van Egmond 2002). Another type of aflatoxin which is found in cow's milk is aflatoxin M1 (Marchese et al. 2018). In a case study, it is found in contaminated milk and various milk products in many countries like Kenya (Lindahl et al. 2018) and Middle East countries (Iran, Turkey, Egypt, Syria) (Rahmani et al. 2018). In a UK-based study, the most affected cancerous tissue found is of colon tissue, showing higher DNA adducts which doubtfully due to aflatoxin exposure into the cells (Marchese et al. 2018; Harrison et al. 1993). The in vitro studies have proved aflatoxin mode of

action by preventing p53 activation e.g., the study on HCT116 colorectal cancer cell line has shown an increase in H2AX phosphorylation, but unable to activate p53, thereby arresting G1 phase of cell cycle (Gursoy-Yuzugullu et al. 2011); another study found aflatoxin exposure activates p53, but there is a higher expression of MDM2 which keeps it inhibited (Kim et al. 2016b).

2.11 Carcinogen from Water

NOCs derived from nitrates are found usually in drinking water due to excessive use of fertilizers in agriculture (Ma et al. 2018). Nitrates are found in vegetables and are also an approved food additive for preserving meat; these nitrates reduce to nitrites and react with amines and amides to produce N-nitrosamine in the gastrointestinal system and eventually increase with more intake of meat (Bedale et al. 2016; Park et al. 2015). NOCs are classified as Group 2B (possibly carcinogenic to human) due to the formation of cancer in rats (Cancer IAfRo 2012); the nitrate intake through drinking water and other dietary sources (vegetables, meat) eventually increases the chances of CRC risk (Espejo-Herrera et al. 2016). Therefore, the level of nitrate content is decided in some countries. Lowa (USA)-based epidemiological study has concluded the safety limit of nitrate (NO_3) to be 50 mg/L in drinking water (van Grinsven et al. 2010). Chlorine is widely used in drinking water to disinfect or kill germs. The use of chlorinated drinking water in surface water sources is high in an Asiatic country like China, which mainly increases the risk of rectal cancer (Doyle et al. 1997; Chen et al. 2005). The by-products generated by the chlorine such as chloroform, carbon tetrachloride (Backer et al. 2000), and trihalomethanes (THMs) (Binnie et al. 2002) are considered as potential carcinogens. To avoid these harmful by-products, chlorine dioxide (ClO2) is used (Vertova et al. 2019) for removing contaminants from water.

Arsenic contamination usually found in groundwater, pond, well, and contaminated soil due to arsenic can lead to CRC mortality in humans (Chen et al. 2015), and mice study has found an alteration in gut flora and metabolism (Lu et al. 2014). Arsenic has been categorized as class I carcinogen (International Agency for Research on Cancer 2004), and it is correlated with various types of cancer formation, and it helps in tumorigenesis by interfering with the ability of immune cells in responding to transformed cells and pathogens (Lemarie et al. 2006). Arsenic contamination has been found in many countries including an Asiatic country like India, where West Bengal-based study has found a high level of arsenic contamination in groundwater and found an increase in inflammatory factors (Roy Chowdhury et al. 2018); also some study has found arsenic in vegetables when irrigated with arsenic-containing water which correlates with the risk of cancer (Bhatti et al. 2013), and the highest arsenic contamination among all was found in spinach. The heavy metals like arsenic and asbestos have been well documented for chronic inflammation (Jaishankar et al. 2014). The asbestos fibers can be found in drinking water mainly contaminated from mining, industries, cement pipes, and water tanks;

although asbestos is banned in some countries, chrysotile (white asbestos) is still in use. Countries like Europe, the USA, Canada, and Australia still use asbestos-made pipes and water tanks, and approximately 15% of the water pipe has asbestos material in North America. The asbestos interferes in colon cancer by inducing oxidative DNA damage and inactivating p53 TSG, which eventually leads to tumor progression and revascularization (Paris et al. 2017). Also, certain study signifies the risk of the stomach and colon-rectum cancer by intake of drinking water containing asbestos in animal studies (Effects IoMCoASH 2006; Kim et al. 2013b). However, there is a limited study to prove their association.

In China, pollution is also one of the major risk factors for an increase in CRC incidence, through burning coal as coal ashes contain heavy metals that pollute the air, water, and food. This uncontrolled pollution due to industries turns villages into "cancer villages" in China (Gao 2013). The other reason for increasing CRC incidence may be due to the high concentration of DDT in spiny-head croaker, trident goby, and pike eel from Lake Tai in Shanghai (Nakata et al. 2005). Also, in some of the industrial areas, shellfish may have a high level of methyl mercury, polychlorinated dibenzo-p-dioxins, and dibenzofurans (Hou et al. 1988). In Shanghai, the consumption of fish is high (~50.6 g/day).

2.12 Role of Lifestyle Factors

The lifestyle factors include healthy eating and regular exercise, which maintain good metabolic activity of the body, reducing ROS production and eventually the risk of cancer. The following are the major lifestyle factors that can contribute to CRC initiation, and similarly, maintaining a healthy lifestyle could eliminate these risk factors.

2.12.1 Obesity

The fast foods, sugary drinks, and fatty foods should be taken in an adequate amount as these high energy-dense foods contribute to obesity, which is a major risk factor for cancer; also the majority of the cancers are due to obesity, unhealthy diets, and lack of physical activity. In the USA, a survey-based study of 2016 has found that 39.8% of adults and 18.5% of youth were obese (Hales et al. 2017), and concerning this, there is a significant increase in death due to various cancers including colon cancer (Calle et al. 2003). The basal metabolic index is used to measure the body health by weight divided by the square of the height in meters (kg/m^2). WHO recommends that a BMI between 25.0 and 29.9 kg/m^2 will be considered as "overweight" and equal to or more than 30 kg/m^2 as "obesity" (No Authors Listed 1998). In the UK, overweight or obesity is the third most cause of cancer, and in England, 22% of men and one-fourth of women are obese, having BMI more than 30 kg/m^2

(Parkin et al. 2011), whereas in China, 2.49% in men and 3.41% of women are obese (Gu et al. 2018). Most of the meta-analysis has identified the relation of obesity with more of colon cancer than rectal cancer (Harriss et al. 2009). The determination of BMI is also correlated with higher expression of prostaglandin E2 which links to a higher risk for CRC (Martinez-Useros and Garcia-Foncillas 2016), and also this association is supported by the large pro-inflammatory cytokines released by adipose tissue which triggers insulin resistance (Olefsky and Glass 2010) and induces oxidative stress (Karin and Greten 2005).

2.12.2 Physical Inactivity

Lack of physical activity leads to obesity. According to IARC, approximately 25% of all cancers are due to obesity and irregular lifestyle which are strongly seen in breast and colon cancer (Wolin et al. 2010). A meta-analysis has shown that there is an increase in the risk of CRC by 18% for every rise of 5 units in BMI, especially in men (Basen-Engquist and Chang 2011). However, there is an increase in proportion in adults who follow physical activity guidelines from 41% in 2006 to 50% in 2012 (Ward et al. 2016). From studies, consistently an inverse relationship between physical activity and CRC is found (Spence et al. 2009). Hence, it is suggested that regular physical activity could prevent the risk of CRC (Clague and Bernstein 2012) as including physical exercise stimulates intestinal peristalsis which reduces retention time of food and carcinogens in the intestinal tract (Wang et al. 2018). Approximately, 15% of cancer can be prevented from physical activity, especially colon (Oruç and Kaplan 2019) and breast cancer (Vainio et al. 2002; Kruk and Czerniak 2013). CRC is mainly caused by comorbid diseases including diabetes, obesity, and pulmonary disease, which account for more than 40% of its cause (Edwards et al. 2014). Including physical activity on a daily or weekly basis (metabolic equivalents, hour/week) can reduce the risk of CRC by comorbids and also can help in managing the disease.

2.12.3 Alcohol Consumption

According to WHO, alcohol consumption is a lifestyle habit that accounts for 5.1% of the global disease burden (World Health Organization 2019) and can contribute to colon carcinogenesis (Bishehsari et al. 2017). Alcohol consumption is higher in men than women in many countries. Its consumption for a long time contributes to the etiology of various cancers (Boffetta and Hashibe 2006), especially gastrointestinal (GI) cancer and organs associated with it. Many individual studies have estimated the quantity of daily alcohol intake and its risk. A meta-analysis suggests more than or equal to 50 g per day of ethanol can contribute to CRC (Cai et al. 2014). In the UK, alcohol consumption is the fourth most common cause of cancer,

which is also a continuously increasing problem (Parkin et al. 2011). The Asiatic based studies gave a different result in terms of alcohol association with gender and colon cancer location. A Korean-based study has shown distal colon cancer in men and rectal cancer in women (Shin et al. 2011). A China-based study has shown 8.7% of cancer deaths in men and 1.1% in women due to consumption of alcohol (Gu et al. 2018).

There are various mechanisms which explain that the consumption of alcohol could lead to CRC initiation or increase the risk. The general metabolism of ethanol/alcohol gets started in the stomach before going into circulation and then directly to the duodenum where there is slow absorbtion and passes out through excretion. The ethanol/alcohol gets converted to acetaldehyde with the help of acetaldehyde dehydrogenase (ADH) enzyme and certain bacteria *Enterobacteriaceae* which have ADH and aldehyde dehydrogenase (ALDH) activity (Elamin et al. 2013). This acetaldehyde further gets converted to acetic acid by ALDH or cytochrome P450 E21 (CYP2E1) (Rossi et al. 2018). The ALDH varies among populations based on ethnic or individual differences, having allelic forms (Na and Lee 2017). Acetaldehyde is highly mutagenic and carcinogenic, so metabolizing it is a very necessary step, but the enzyme metabolizing it (ALDH) has an allelic difference in population, e.g., approximately 40% of Japanese, Korean, or Chinese population have carriers for ALDH 2*2 allele in heterozygous form, which codes for minimum activity of enzyme (Seitz and Stickel 2010). Adding on, acetaldehyde accumulation causes DNA damage in the digestive tract, and it is considered as Group 1 carcinogen by IARC (IARC 2009; Secretan et al. 2009; World Health Organization 2000). Acetaldehyde also gets metabolized by the microbiome in the colon but gets accumulated due to the low activity of ALDH in the colon (Malaguarnera et al. 2014). The accumulation of acetaldehyde further contributes to the formation of colonic polyps and increases the risk of CRC. Its accumulation further also causes adducts in DNA (Heymann et al. 2018). CYP2E1 enzyme initially metabolizes the ethanol and produces reactive oxygen species (ROS) in large amount (Rao and Kumar 2016). Hence, the accumulation of acetaldehyde and ROS during ethanol metabolism forms adducts in DNA which further can cause CRC. Alcohol consumption and its metabolism affect multiple consequences leading to genetic abnormality, epigenetic deregulation, abrupt cell signaling, and deregulated immune response, resulting in cancer and providing favorable environment for the tumor growth (Rossi et al. 2018).

2.12.4 Smoking

In the UK, controlling tobacco has become the main objective for the past 50 years, due to the topmost rank in the cause of cancer (Parkin et al. 2011). The Asian Working Group stratified smoking as a predictive factor for advanced neoplasia based on scores related to age, gender, and smoking (Asia-Pacific Colorectal Screening Working Group for CRC) (Li et al. 2016; Yeoh et al. 2011). In China,

CRC-related deaths due to smoking attribute both mortality and incidence to 8.7% in men and 0.4% in women (Gu et al. 2018). Smoking cigarettes increases the risk of CRC, and it has a strong association with rectal cancer than colon cancer (Liang et al. 2009). Tobacco content has many toxic effects due to many harsh compounds present in smoke such as PAHs (BaP), N-nitrosamines such as 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK), and N′-nitrosonornicotine (NNN) (Petti 2009), among which NNK, NNN, and BaP are considered as Group 1 carcinogen (International Agency for Research on Cancer 2010b). Smoke also consists of heavy metals (Ni, Cd, Cr, and As) and carbon monoxide that cause inflammation, immune changes, genetic alterations, and oxidative damage (Qiu et al. 2017). These carcinogenic compounds can easily penetrate the alimentary tract or the circulatory systems and form DNA adducts, inducing free radicals that can promote tumor genesis in CRC. Nicotinic acetylcholine receptors on cancer cells induce the release of growth factors such as vascular endothelial growth factor (VEGF) which increases tumor angiogenesis and cancer growth (Zhao 2016). Another mechanism in which colon cancer cells undergo epithelial to mesenchymal transition (EMT) is by expression of COX-2 and activating prostaglandin enzyme 2 (PGE2), which also increases mesenchymal transcription factors and further invasion (Dinicola et al. 2018). Adding on, cigarette smoke also induces epigenetic alterations in inflammation (Zong et al. 2019).

2.13 Immune Status

As one individual is different from the other, so as cancer initiation differs from patient to patient due to unique characteristics of the person (including genetics and immune response) or influential environmental factors (diet, lifestyle, toxins, and microbiome). It has been apparent that the pathogenic process of colorectal cancer is a multistep process specified by the “unique disease principle” (Ogino et al. 2012). To know about these uniqueness in a population among people with the same disease but affecting differently, a collaboration of different expertise get into some conclusion. The molecular pathological epidemiology (MPE) and immunology-MPE study helps clinicians and researchers to understand this difference in disease etiology. It helps to understand tumor-immune interactions during the different stages of CRC initiation and progression. Epidemiological studies, molecular pathology, and immune response after exposures (exposome) (Ogino et al. 2018) can nail down the role of the immune system in CRC pathogenesis.

Some studies has concluded that the incidence and mortality due to colon cancer is more in men than women. This difference is explained in immunology-MPE study (Campbell et al. 2019), which is previously thought due to sex hormone differences, but by analyzing RNA sequence data from TCGA for colon cancer, no significant differences are seen between tumors of both genders for gene expression. Rather, there is a difference in drug and xenobiotic metabolism via P450 pathways which is probably more definite in women. This hypothesis when

replicated into study, found that there is no drug metabolism pathways associated with survival in men, however the chemotherapy targeting this pathway increased the 10-year survival rate in women (Campbell et al. 2019; Lopes-Ramos et al. 2018).

Normally, diet influences our immune system by gut microflora such as *Bifidobacterium* spp. and *Lactobacillus* spp. which helps in maintaining a healthy gut by the production of antiapoptotic factors such as SCFAs, affecting colonocyte's metabolism, signaling, and health (van der Beek et al. 2018). Dysbiosis in this could increase the risk of colorectal carcinogenesis. Certain diet components such as, carbohydrate constituents inulin and oligofructose which activates the secondary lymphoid organ (GALT) leading to production of IL-10 and natural killer (NK) cells, also production of SCFA and lowering the risk of CRC (Watzl et al. 2005). Similarly, the diet which includes fiber-containing fruits, vegetables, and wholegrain is associated with a lower risk of CRC (Ströhle et al. 2007). In contrast, Western dietary foods like processed red meat promote inflammation and alter the gut microbiome by decreasing *Firmicutes* spp. and increasing pathogenic *Enterobacteriaceae* spp. in colorectal cancer (Liu et al. 2018). Alcohol intake also increases pathogenic *Clostridium* spp. (Capurso and Lahner 2017). *Fusobacterium nucleatum* is an anaerobic gram-negative bacteria that contribute to colorectal carcinogenesis by modulating host immune response and inducing tumorigenesis by activating signaling pathways that contribute to it, proved in animal and human studies (Kelly et al. 2018). The formation of CRC is a multistep process forming from the contribution of molecular alterations, influence from the diet, environmental factors, microbial exposure, and immune response. Fusobacterium enrichment in CRC has been found in the metagenomics study. A cohort-based comparative study has found a high number of *F. nucleatum* species in CRC samples of US patients compared to Japanese CRC samples (Mima et al. 2015), and its expression is also linked with high microsatellite instability (MSI-H) and CpG island methylator phenotype (CIMP) in colorectal cancer (Tahara et al. 2014). *F. nucleatum* inhibits immune activation by inhibiting T-cell proliferation and inducing T-cell apoptosis. It also regulates (miRNA21) expression and releases IL-10 and prostaglandin E2 which inhibits antigen presentation by dendritic cells and suppresses T-cell-mediated antitumor response (Nosho et al. 2016).

2.14 Conclusion

Based on the data available in the literature, increasing trend of CRC in Asiatic countries is mainly due to changing dietary habits, switching to Western dietary habits, and other lifestyle factors. Most of the Asian countries like China, Japan, and Korea have inclined toward the consumption of more meat than vegetables and fruits. The etiology difference is still the main cause of CRC incidence in various countries, which influences gender as well as the site of cancer. For example, in

Korea, men are more affected by CRC than women, and similarly, obesity increases the risk of getting colon cancer, whereas smoking is associated with rectal cancer. These differences are very well understood by looking through the dietary habits in the various country, suggesting Western diet habits should be replaced with MeD which helps in decreasing the risk of CRC. The risk factor for CRC can also be manageable by, making small changes in cooking method by avoiding grilling, barbecuing meat at high temperature and deep frying the food to prevent the formation of carcinogenic compounds such as HCAs, PAHs, and NOCs. It is advisable to minimize barbecuing the food which produces the highest amount of carcinogens compared to other cooking methods; also the oil used in cooking produces aldehyde which releases maximum in deep-frying which can be replaced with using palm oil and stir-frying. Adding on, salt is highly used in preserving food mainly in packed foods, and in some countries, a high amount of salt is added in pork; this should be avoided as it damages the intestinal epithelial cell. Instead, the salt-containing leafy vegetables can be used such as spinach. It is suggested in many studies to take a good amount of vegetables and fruits in the diet. Indeed, our food is only as healthy as it is grown, but the biggest concern arises when that healthy vegetable consists of silent harmful compounds like pesticides. These pesticides are used in agriculture from where we obtain our food. This is an unavoidable fact that pesticides save our crops from getting damage by insects, but only the small percent of pesticides kill the insects and rest gets mix into the soil and water, resulting into the food chain and affecting various molecular pathways leading to cancer. Similarly, AFB1 mainly seen in the agriculture field of countries like Southeast Asia and China has chances to be found in food and beverages; the more concern is its presence in mother's milk and cow milk. Other environmental pollutants that pollute our environment and water, such as chlorine which is a common disinfectant used in water, increase the risk of rectal cancer; similarly, arsenic influences the immune cells. In the USA, asbestos is used in water pipes, which is a heavy metal and has chances to form adduct in DNA and eventually CRC.

It is an unavoidable fact that we are the result of lifestyle we adopt for; lack of physical activity can lead to obesity which mainly makes IGF resistant, and insulin resistance is associated with the risk of getting CRC. Therefore, adequate hours of exercise and including little physical activity on daily basis can reduce the risk of CRC and also can help in proper bowel moment. Besides, other habits such as drinking and smoking should be avoided. Also, there are differences in etiology in Western and Asian countries because of the involvement of gut flora and immune response. Dietary habits and lifestyle factors maintain the balance between microbiomes and boost our immune response. Therefore, by reviewing all the risk factors related to CRC and their burden in different countries can be manageable by making small changes in our life style and dietary habits.

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Chapter 3
Diet and Colon Cancer: A Comprehensive Review

Rewa Kulshrestha and Soumitra Tiwari

Abstract Cancer is a global issue, and among cancer, colorectal cancer (CRC) is the third most frequent cancer in men, after lung and prostate cancer, and is the second most frequent cancer in women after breast cancer. Dietary factors account for nearly half of all CRC cases. Diet has a paramount role in the development of CRC. In the past few decades, findings from widespread epidemiologic and experimental exploration have linked ingestion of numerous foods and nutrients to the risk of CRC. For example, consumption of fiber, whole grain, and calcium has been associated with a lower risk of colorectal cancer and red meat and processed meat with an increased risk of CRC. In light of the above findings, precautionary actions, which includes adapting to better dietary patterns and lifestyle, are among the better approach to mitigate the global burden of CRC. Considering the importance of diet, this chapter aims to summarize and discuss the pertinent epidemiologic data that links dietary behavior and colorectal cancer. The aim of this review is to discuss all the foremost elements associated with diet that may have a role in CRC. Also, the latter half of the review focuses on latest epidemiologic and clinical trial evidence in treatment of CRC. In the end of the review, we aim to propose particular dietary suggestions, which can be used for dietary modification to deal with CRC prevention.

Keywords Colorectal cancer · Dietary fiber · Dairy lcroducts · Meat

3.1 Introduction

Cancer is a multifactorial heterogeneous form of ailment, typically resulting from key factors such as lifestyle, genetic, and environmental factors. It is one of the foremost causes of morbidity and mortality worldwide. Among cancers, colorectal

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cancer (CRC) is the third most frequently diagnosed cancer form and the fourth prominent reason of death associated with the cancer globally (Ferlay et al. 2013). In developed countries like the USA, in the year 2019, approximately 145,600 new cases of CRC and 51,020 deaths were estimated to occur (US Preventive Services Task Force 2008). Although screening reduces the incidence and mortality from CRC (US Preventive Services Task Force 2008; Zauber et al. 2008; Gallaher and Trudo 2017), the routine use of screening has been a limiting factor due to resource constraints. Thus, preventative measures, which include dietary and lifestyle alterations, are therefore an appealing strategy to reduce the CRC global burden. Over the past few decades, migration studies and prospective cohort studies have established the important effects of diet and lifestyle in the development of CRC. Approximately 50–60% of incident cases of CRC in the USA are estimated to be attributable to modifiable risk factor (Holme et al. 2014). Various evidence have been reported that link the association of diet and CRC (Bartsch et al. 1999). For example, in one of the past studies, authors projected 35% of mortality related to cancer and 90% of stomach and large bowel cancer-related deaths related to the factors associated with diet (Gupta et al. 2014). In the last decade, abundant research and clinical trials (RCTs) have been conducted to recognize impending dietary factors to CRC risk (Doll and Peto 1981; Garland et al. 1985). Colossal human data has been gathered so far with diverse studies and human trials and does indicate promise of dietary chemoprevention (Newmark et al. 1984).

In this review, an endeavor is made to analyze key factors associated with diet that plays a role in the CRC, and addressing the most evidence associated with epidemiologic and clinical trial evidence**.** In addition, built on amalgamation of the evidence, we propose key suggestions for clinicians who may seek advice on the type of diet for cancer prevention. In the coming section, we would lay emphasis on the role of nutrients in CRC.

3.2 Role of Nutrients in CRC

Diet is one of the most acknowledged factors causative cancer etiology. Among the numerous dietary components, the role of micronutrients has engrossed much consideration of the scientific community worldwide. Below section addresses the importance of different food micro-molecules on CRC. Micronutrient addition via route of diet may protect an individual from the onset and prevention of CRC. Summary of the role of nutrients and food in CRC prevention is presented in Fig. 3.1.

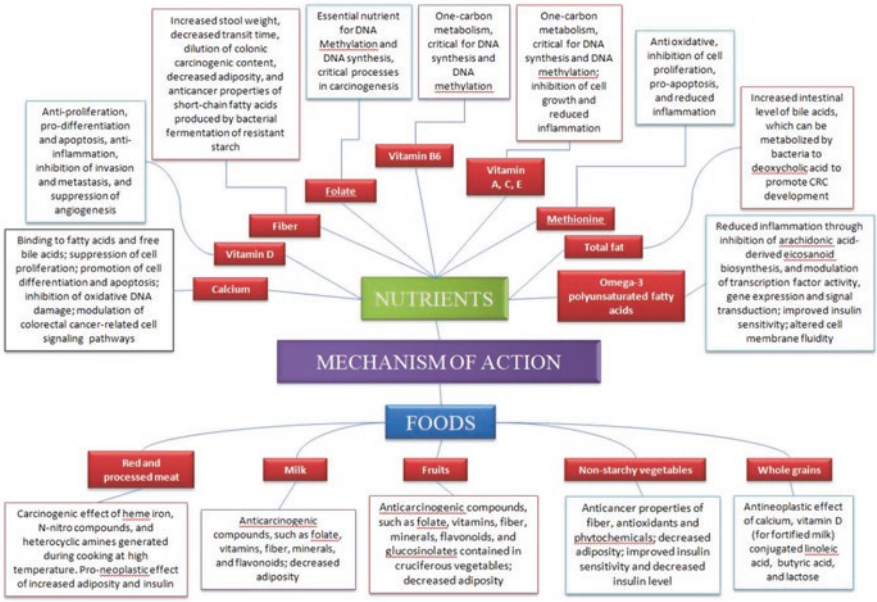

Fig. 3.1 Role of nutrients and food in CRC prevention

3.3 Calcium

Calcium is present in a variety of foods, including whole-grain cereal products, leafy vegetables, legumes, nuts, and more abundantly found in dairy products such as milk, yogurt, and cheese. Past literatures suggest that the calcium has a protective effect against the many types of cancers. With reference to CRC, relatively high intakes of calcium have been found to reduce the risk associated with the CRC (Doll and Peto 1981). The ability of ionized form of calcium to form insoluble soaps with tumor-promoting free fatty acids and bile acids in the colonic lumen led to the hypothesis that calcium was antineoplastic.

Nutrients that both increase and decrease risk may coexist in the same food commodity (Garland et al. 1985; Newmark et al. 1984; Potter 1996; Martínez 2005; Park et al. 2007). Evidence for this intra-food interaction is illustrated by the differences in risk estimates found for milk and dairy products, which vary in fat, protein, and calcium content. Mouse studies have indicated that calcium and vitamin D have potential antineoplastic effects in the colon (Wargovich and Lointier 1987). Epidemiological studies also support the findings (McCullough et al. 2003a).

To summarize, major evidence suggests that a person with higher intake of calcium (700–1000 mg/day) has a lower CRC risk. Thus, it is prudent to increase the calcium uptake in the diet.

3.4 Vitamin D

Vitamin D belongs to a group of steroids with a broken ring recognized as secosteroids. Among different forms of vitamin D, vitamin D_3 (cholecalciferol), which is produced in the human skin, and vitamin D_2 (ergocalciferol) typically derived from the plants are the most significant. The most active metabolite of vitamin D is 1,25-dihydroxyvitamin D ($1,25(OH)_2D_3$, calcitriol). It is synthesized in an exceedingly regulated multi-step process (Jenab et al. 2010).

The vitamin D role in CRC prevention was first hypothesized by Frank and Cedric Garland established on the basis of ecological studies (Garland et al. 2009). These authors proposed that the inverse relation between solar radiation (latitude) and CRC mortality (Garland et al. 2009). Post this finding, several studies have focused on the association between vitamin D status and colorectal adenoma and carcinoma incidence or mortality. Some of these studies and findings are discussed below.

Epidemiological studies indicate that deficiency of vitamin D upsurges the occurrence of colon cancer and also has a negative impact on the survival of colon cancer patients (Giovannucci 2005). The ability of 1,25D to interfere with signaling and to ameliorate inflammation is likely to contribute to its anticancer activity. In agreement, vitamin D_3 level appears to be a crucial determinant for the development and advancement of colon cancer, and supplementation with vitamin D_3 is effective in suppressing intestinal tumorigenesis in animal models. Figure 3.2 depicts antitumor action of vitamin D_3.

Certain studies do suggest that vitamin D_3 can lower the incidence of colorectal cancer by 50% (Giovannucci 2005; Martínez et al. 1996; Lin et al. 2005; Heilbrun

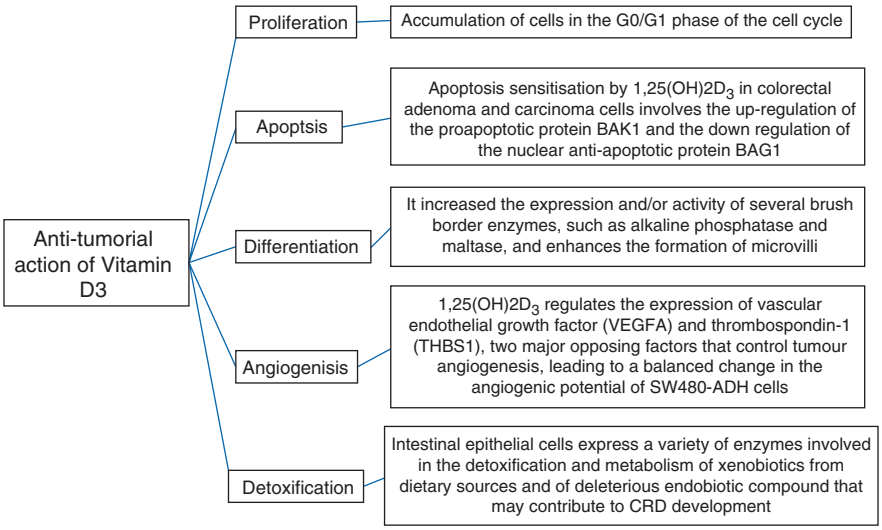

Fig. 3.2 Antitumor action of vitamin D_3

et al. 1985). These studies are consistent with the inverse correlation between dietary vitamin D_3 intake or sunlight exposure and human colorectal cancer. Several studies confirmed that increasing vitamin D_3 lowers colon cancer incidence and that sufficient levels of vitamin D_3 are associated with better overall survival of colon cancer patients. Vitamin D and its analogues reduce the growth of colon cancer xenografts and impede tumorigenesis in numerous genetic models of intestinal cancer (Garland et al. 2009; Giovannucci 2005; Martínez et al. 1996; Lin et al. 2005; Heilbrun et al. 1985). In agreement, dietary initiation of colon cancer in rodents, a model of sporadic colon cancer, has been shown to be prevented by supplementation with vitamin D_3 (Martínez et al. 1996).

The anti-inflammatory and immune regulatory impact of the vitamin D uptake is predominantly captivating and may arbitrate its role in the infectious disease like vascular, neurologic, and autoimmune. For example, in mice (colitis), higher inclusion of vitamin D in the diet attenuated inflammation, signifying that vitamin D may have an imperative role in inflammation-associated carcinogenesis. In one of the studies, high plasma 1,25(OH)D was linked with lower risk of tumors associated with CRC with high lymphocyte counts, but not low-count tumors (Lin et al. 2005; Heilbrun et al. 1985; Kampman et al. 2000).

To summarize, convincing data from various reports support the chemopreventive impact of vitamin D against CRC development (Um et al. 2019).

3.5 Dietary Fiber

Genetic variation and environmental exposure (including diet and physical activity) are one of the two foremost causative factors swaying the manifestation of colon cancer (Wargovich and Lointier 1987). Thus, colon cancer may be extremely pliable to prevention via route of dietary practice, and in turn, dietary carbohydrates might play a critical role.

Dietary fibers are fundamentally carbohydrates. Carbohydrates can be parted into two basic groups centered around digestibility in the GI tract. For example, simple carbohydrates, which represent the first group (starch and simple sugars), can be hydrolyzed by enzymatic reactions and absorbed in the small intestine. Complex carbohydrates (lignin, cellulose, and pectin) are resistant to digestion in the small intestine and undergo bacterial fermentation in the colon are part of second group. These complex carbohydrates, collectively referred to as dietary fibers in our discussion, are majorly found in plants. Countless research in this area indicates association between high dietary fiber intake and a low CRC. To further support this, the US FDA has approved health claims supporting the role of dietary fiber in cancer prevention (McCullough et al. 2003a).

The providence of fiber in the colon is related to the microbes present in the colon and the fiber characteristics like highly fermented or poorly fermented. For example, fiber like pectin and oats are highly fermented, and cellulose and wheat bran may be poorly fermented. Dietary fiber type impacts the composition of

microbial within the lumen of the gut. To further understand it, certain fibers like inulin found to occur in onion and garlic stimulate *Bifidobacteria* growth and inhibits or restricts the growth of pathogenic bacteria.

In one of the past studies, dietary xylo-oligosaccharides were found to associate with the diminution bacteria *Faecalibacterium prausnitzii* (Kim 2000; Slavin 2001). This provides perceptible indication that dietary carbohydrates can alter the gut microbiota and, subsequently, its ability to impact the colon.

Numerous studies have also reported that low rates of colorectal cancer in Africa were due to the high consumption of dietary fiber. Hypothesis mechanism proposed that reduced concentrations of intestinal carcinogens are due to the bacterial fermentation of resistant starch to short-chain fatty acids (SCFAs) (Trock et al. 1990). Butyrate, the major SCFA produced by colonic fermentation, is the preferred energy source for colonocytes and may enhance apoptosis and inhibit proliferation of cancer cells. In addition, SCFAs have also been known to have immune modulatory and anti-inflammatory effects, acting to influence gastrointestinal and perhaps systemic health.

Fiber can act against cancer directly or indirectly (Maldonado et al. 2016). Direct mechanisms involve aggregating fecal bulk (diluting carcinogens), increasing transit time through the colon, and direct binding of carcinogens like aflatoxin B_1, HAAs, polycyclic aromatic hydrocarbons (PAHs), etc., which otherwise can alter DNA (Slavin 2001). Indirect mechanism can be ascribed to restricted or total degradation of dietary fiber. It also embraces alteration of the enzymatic activities of bacteria within the intestinal vegetation and production of short-chain fatty acids (SCFAs) (like acetic, butyric, and propionic – carbon dioxide, hydrogen, methane) and water, leading to the fluctuation of the colonic pH (Slavin 2001; Trock et al. 1990).

The prebiotic fiber supports the growth of beneficial bacteria in the gut, which supposedly enhance the immune system and help indirectly in supporting better food digestion. Some of the foods which contain prebiotic fiber are onion, garlic, leeks, beans, lentils, oats, etc. Allium compounds in garlic and onions are believed to be predominantly effective at preventing bowel cancer. Despite methodology restraint and disparities across studies, substantial evidence exists for inverse risk associations with vegetable and fruit intake for cancers. To support this hypothesis, a study carried out in Korea on 270 cases defines that protective associations were noted for fiber on assessment of intakes of nutrients and food groups by a semi-quantitative food frequency questionnaire and analysis by the logistic regression model (Park et al. 2000). Figure 3.3 presents a proposed mechanism of primary action related to dietary fiber consumption, gut microflora, and colon cancer. Also, Table 3.1 depicts intervention studies using high fiber as a chemopreventive strategy.

In summary, it is clear that dietary fiber intake has a positive role in the mitigation of CRC risk.

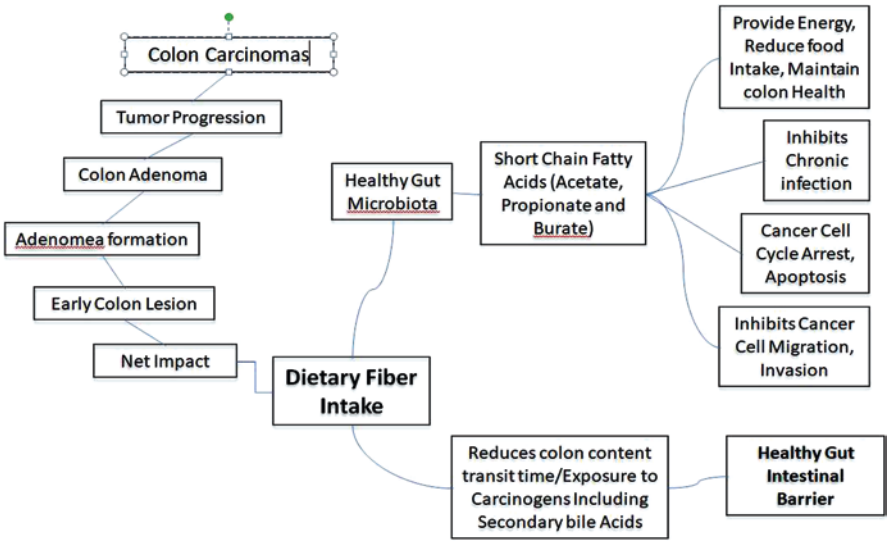

Fig. 3.3 Proposed mechanism of action related to dietary fiber consumption, gut microflora and colon cancer

3.6 Foods

Consuming foods results in multiple and complex interaction among multiple nutrients, which can provide supplementary understanding of the importance of dietary factors in CRC. Here, we limit our discussion down to specific food which is reported in literature with the paramount epidemiologic evidence in CRC.

3.7 Meat

In its methodical assessment to ascertain relationship between diet and cancer, the World Cancer Research Fund (WCRF) recognized that there is a substantial indication that meat (including red and processed) increases risk of CRC (Demeyer et al. 2008). To further support this statement, a meta-analysis indicated an approximately 20% higher risk of CRC per 100 g/day increase in red meat and 50 g/day increase in processed meat (Demeyer et al. 2008). The risk is found to be increased linearly with higher intake of red and processed meat. Similar findings have been reported for colorectal adenomas by various other authors (Santarelli et al. 2008; Corpet 2011; de Vogel et al. 2005; Domingo and Nadal 2017; Goldbohm et al. 1994).

Numerous mechanisms have been found that may further reinforce the argument that red meat ingestion increases the risk of CRC (Demeyer et al. 2008; Santarelli et al. 2008; Corpet 2011). Meat is a major source of sulfur-containing amino acids and saturated fats. In the meat which is processed, the presence of inorganic sulfur

Table 3.1 Intervention studies using high fiber as a chemopreventive strategy

Case diagnosis	Sample size	Country	Type of study	Duration	Total fiber intake (g/day)	Outcome	References
Familial adenomatous polyposis, total colectomy, and ileorectal anastomosis	58	USA	RCT	4 years	11.3	High-fiber protective only if 11 g/day	DeCosse et al. (1989)
Previous colorectal adenomas	100	USA	RCT	9 months	14.4–17.5	52% decrease with high fiber/36% decrease with high fiber	Alberts et al. (1996, 1997)
Previous colorectal adenomas	201	Canada	RCT	2 years	35	Intention-to-treat, no effect Sub-analysis in those with substantial dietary counseling, nonsignificant 50% reduction in women and 90% increase in men with high fiber	McKeown-Eyssen et al. (1994)
Previous colorectal adenomas	424	Australia	RCT	4 years	NA	Low-fat, high-fiber decreased recurrence of 0.10-mm adenomas	MacLennan et al. (1995)
Previous CRC	17	USA	Single arm, uncontrolled	8 weeks	30.9	Overall 22% decrease compared with baseline	Martínez et al. (1998)

as a preservative can be also found. Various studies suggest that oxidative stress can be induced by heme iron in red meat leading to proliferation and endogenous formation of N-nitroso compounds (NOCs). These compounds are known to be potent carcinogens primarily located in the gastrointestinal tract (Santarelli et al. 2008).

Additionally, various reports have suggested that meat cooking at high temperature can also lead to other mutagens, including heterocyclic amino acids (HAAs) and polycyclic aromatic hydrocarbons (PAHs). High consumption of heme iron (but not other forms of iron), NOCs, HAAs, and PAHs have all been associated with

increased risk of colorectal tumors (Corpet 2011). HAA consumption creates chemical leading to the DNA modification, typically recognized as DNA adducts. The structures of two HAAs commonly found in foods and their DNA adducts are shown in Fig. 3.4. DNA adducts formation is largely considered to be a necessary (although not sufficient) occurrence for tumor formation. A recent study in which dietary HAA intake was estimated for three HAAs found significant positive correlations between intake of several HAAs and colorectal tumors (de Vogel et al. 2005).

Based on the human study trials, in the year 2011, joint study conducted by the World Cancer Research Fund/American Institute for Cancer Research (WCRF/AICR) supported the idea that the intake of red meat and processed meat (smoked, cured, salted, etc.) can cause higher risk of colon and rectal cancer (Domingo and Nadal 2017). Also, a working group linked to the International Agency for Research on Cancer (IARC), multiple scientist span over different countries evaluated over 800 epidemiological studies to understand the association between colorectal cancer and red meat and processed meat (Domingo and Nadal 2017; Goldbohm et al. 1994). They concluded that consumption of processed meat is "carcinogenic to humans" with the evidence mostly related to CRC (Goldbohm et al. 1994; Willett et al. 1990; Turner and Lloyd 2017; Abu-Ghazaleh et al. 2020; Clinton et al. 2020).

In summary, coherent with their mutagenic impact, processed meat and heme iron have been more persistently associated with the higher probability of colorectal neoplasia.

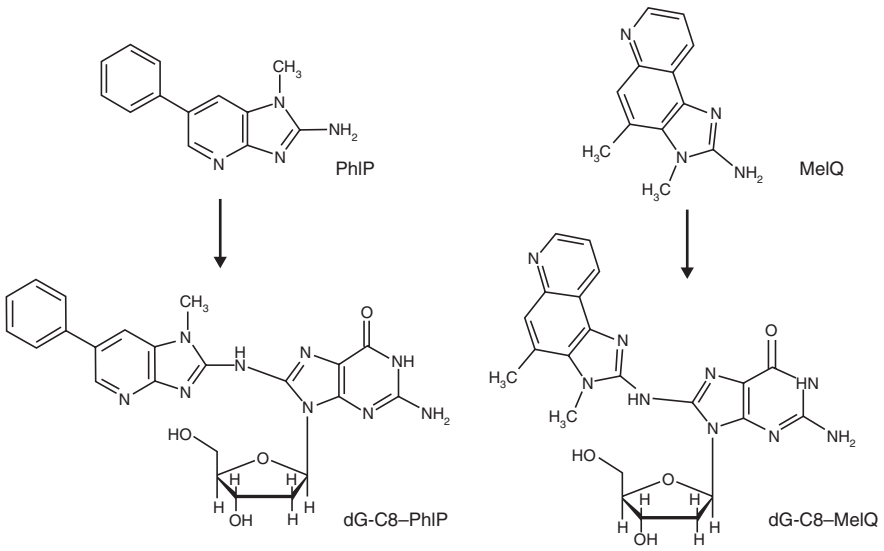

Fig. 3.4 Structure of two foodborne HAAs and their DNA adducts (Santarelli et al. 2008)

3.8 Dairy Products

A number of constituents in dairy foods have been reconnoitered for their chemopreventive potential. Among them, calcium and vitamin D have received the most attention. Additionally, lipids in the dairy fat, for example, conjugated linoleic acid (CLA) and sphingolipids, as well as dairy proteins, particularly the whey proteins, have also been explored (Clinton et al. 2020).

Various reports do indicate the protective role of dairy products in CRC due to the presence of higher amount of calcium along with other micronutrients (Holt 1999, 2008). The role of calcium is already addressed in the previous segment of this article.

Apart from calcium, other micronutrients like conjugated linoleic acid (CLA) can positively contribute to the antineoplastic activity. To further support this theory, various studies in the past have been conducted. In animal studies, inclusion of CLA has inhibited CRC. In human studies, similar findings have been reported. Apart from CLA, butyric acid, a SCFA, has also been found to be protective against CRC (Bostick et al. 1993). Table 3.2 depicts the conjugated linoleic acid content of various dairy products.

To summarize, various evidences suggest the positive impact of consumption of dairy products on CRC.

3.9 Fruits and Vegetables

Fruits and vegetables can have protective impact against CRC because of high levels of several phytochemicals (refer to Fig. 3.5).

Table 3.2 Conjugated linoleic acid content of dairy products

Dairy	mg/g fat
Milk (homogenized)	5.5
Butter fat	6.1
Condensed fat	7
Cultured buttermilk	5.4
Butter	4.7
Sour cream	4.6
Ice cream	3.6
Low-fat yogurt	4.4
Custard-style yogurt	4.8
Plain yogurt	4.8
Frozen yogurt	2.8
Medium cheddar	4.1
American processed cheese	5

Adapted from MacDonald (2000)

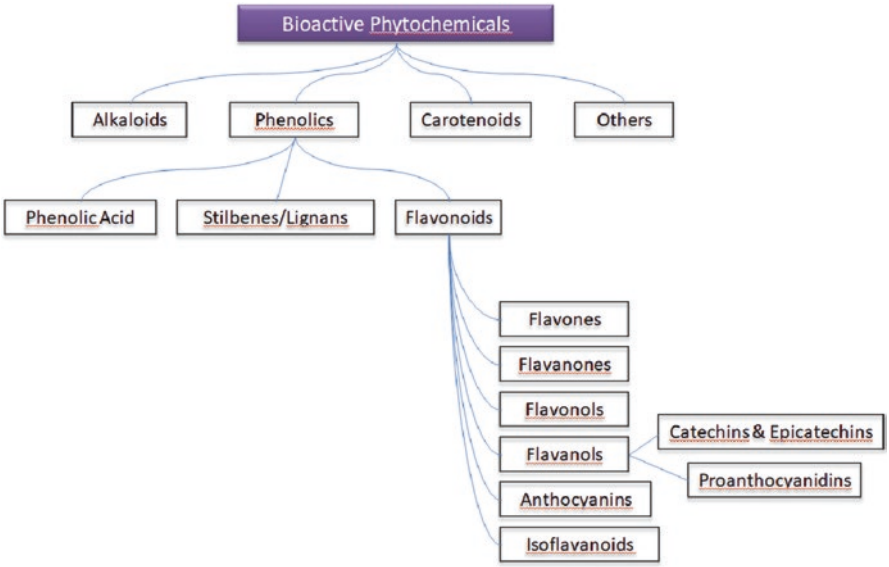

Fig. 3.5 Bioactive chemicals in fruits and vegetables having chemopreventive activities

In one of the meta-analysis studies, a substantial non-linear association was unearthed between fruit and vegetable intake and CRC incidence. These studies suggested the greatest risk reduction with increase in intake of fruits up to about 100 g/day and vegetable intake to about 100–200 g/day and also found diminutive evidence for supplementary reduction with higher intake (Allen 2018). This non-linear relationship may explain the discrepant findings, as in some studies, the lowest level of intake was already above the beneficial effect range. In addition, there are also studies that support the claim that higher intake of fruit and vegetable during adolescence and midlife, independent of adult diet, may provide supplementary advantage, suggesting that exposures over the life cycle can play a critical role in CRC development.

Among vegetables, cruciferous vegetables are of specific significance owing to the presence of higher content of glucosinolate. The multidimensional antineoplastic activities of Isothiocyanates (ITCs) and Indole-3-carbinols (I3Cs) have been reported in various animal studies. An impending advantage of elevated intake of cruciferous vegetable/ITC levels on colorectal neoplasms has also been reported in the past studies. For example, in one of the meta-analyses, almost 16% reduction of risk associated with CRC was reported comparing the highest to the lowest categories of intake of cruciferous vegetable (Wiseman 2008; Steinmetz et al. 1994). Genetic studies also provide the ancillary evidence, in which genetic variations in glutathione S-transferase, a critical enzyme in ITC metabolism, modify the association of cruciferous vegetables or ITCs with colorectal tumors. Contemplating the prominence of gut microbiota in glucosinolate metabolism, auxiliary exploration is indispensable to unearth the potential interplay between genetic susceptibility, gut

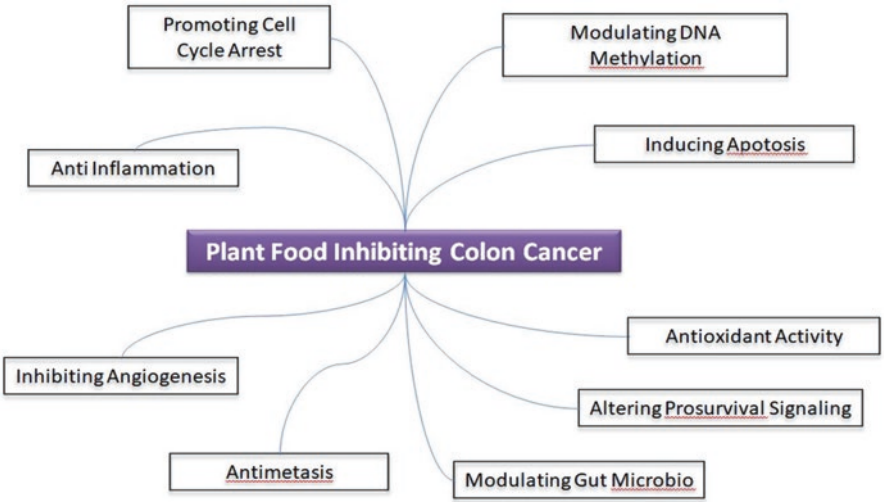

Fig. 3.6 The mechanisms of protective effects of plant foods on colon cancer

bacterial composition, and dietary intake in determining CRC risk (Koushik et al. 2007; McCullough et al. 2003b; Slattery et al. 1998; Willett 2008; Nelson 2001).

To summarize, higher intake of fruits and vegetables is associated with lower risk of CRC (Block et al. 1992) (Fig. 3.6).

3.10 Whole Grain

In contradiction to refined grains that come with existence of endosperm, whole grains additionally have bran and germ (Fig. 3.8). These are known to be rich sources of various substances with anticancer properties. Past studies support the indication that higher intake of whole grain can lower the risk associated with CRC (Larsson et al. 2005; Egeberg et al. 2010). However, at this conjecture, it is imperative to discuss a particular challenge of studying whole-grain intake in relation to cancer studies. It is challenging for precise measurement of whole-grain consumption owing to the wide variation in whole-grain content among products and absent universal standards for whole grains or whole-grain products. To address this impending concern, a circulating biomarker of whole-grain intake, alkylresorcinol, has been used for the blood plasma measurement.

Alkylresorcinols, phenolic lipids present in the wheat bran, are not impacted by processing of the food and thus can be precisely quantified in blood plasma with reasonable validity and reproducibility. In one of the case-control studies, the highest quartile of plasma alkylresorcinol was associated with decreased risk of distal colon cancer, but not proximal colon or rectal cancer. Few research findings have

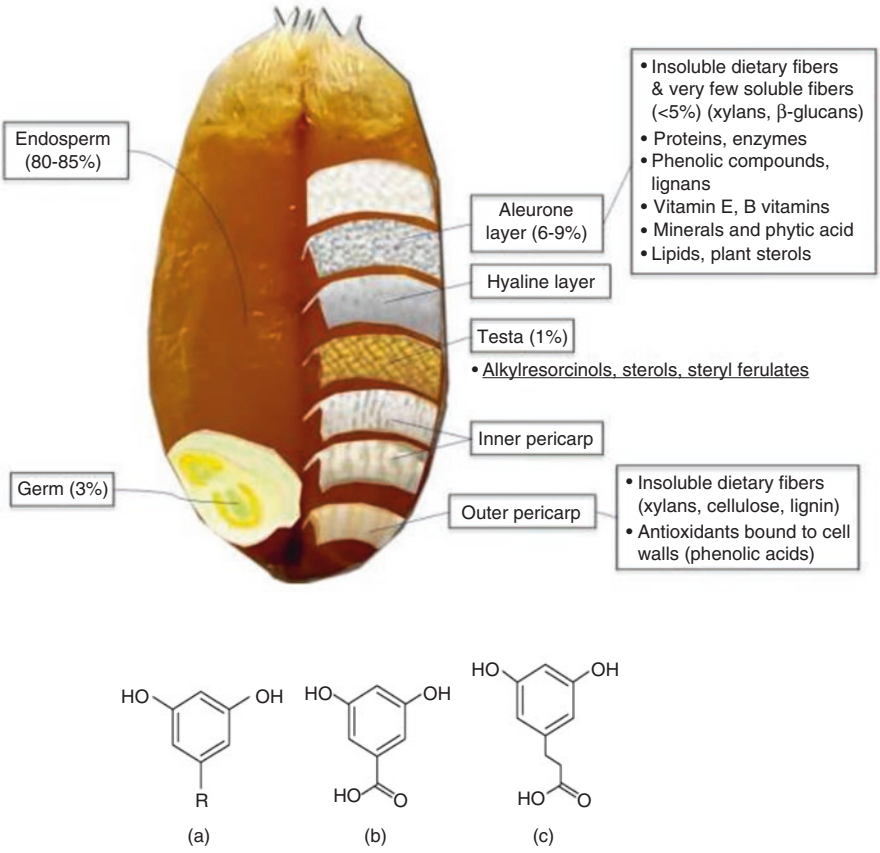

Fig. 3.7 Schematic structure of different layers present in wheat and Basic structure of alkylresorcinols (**a**), and the two main plasma and urinary metabolites, 3,5-dihydroxybenzoic acid (**b**) and 3,5-dihydroxyphenylpropianoic acid (**c**). For the most abundant alkylresorcinols in cereals, R = $C_{17}H_{35}$–$C_{25}H_{51}$ (Pan et al. 2018)

shown that phytochemicals in wheat bran (WB) may be associated with anticancer activities (Fig. 3.7).

In one of the animal studies carried over by Reddy's group, it was found that the lipid fraction of WB (WB oil) had the inhibitory effect on tumorigenesis in the azoxymethane (AOM)-induced colon tumor model in rats (Reddy 1999). Using human HCT-116 and HT-29 colon cancer cells as guiding assays, it was also found that subfraction containing 5-n-alkylresorcinols (ARs) had the strongest inhibitory effect on the proliferation of human colon cancer cells (Tieri et al. 2020). ARs are 1,3-dihydroxybenzene derivatives with an odd-numbered alkyl chain at position 5 of the benzene ring.

In summary, based on the evidence, higher intake of whole grains helps in the reduction of CRC.

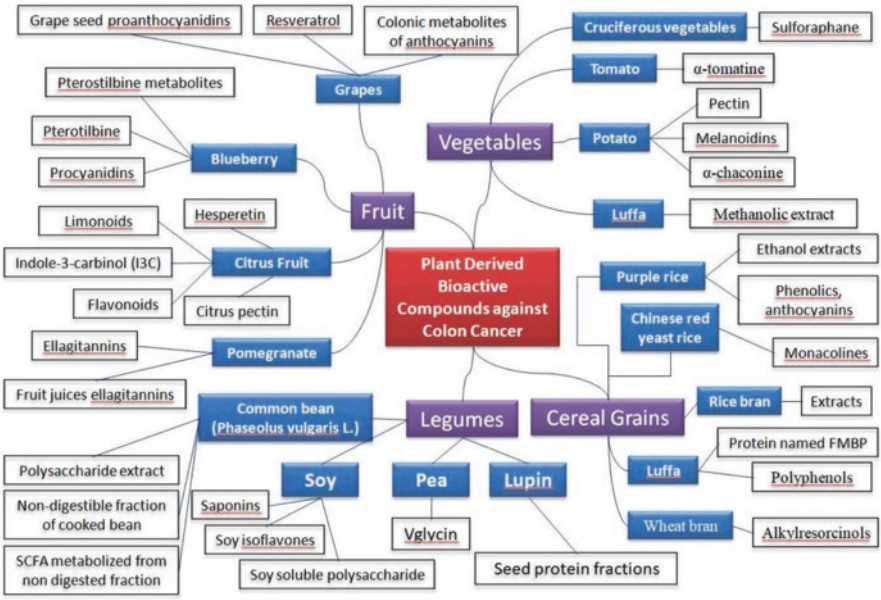

Fig. 3.8 Representation of bioactive compounds present in plants against colon caner

3.11 Dietary Pattern

Incipient information advises that a dietary pattern may demonstrate stronger connotations with CRC risk compared with specific nutrients or food. Various studies have focused on the different patterns of diet representing combination of highly correlated foods consumed in a certain population based on the approach based on dietary recommendations or data-driven approach by factor or cluster analysis (Malila et al. 1998; Nelson 2001). The data-driven approach has acknowledged at least two general patterns. One of the patterns features high consumption of processed and red meats, refined grains, soda, and sweets typically witnessed in the Western diet, and the prudent pattern takes into account high intakes of fruits, vegetables, fish, poultry and whole-grain products. Studies relates to higher risk of CRC among the population which relates to Western pattern diet whereas the prudent pattern, has been less consistently associated with lower risk.

Thus, in conclusion, it would be prudent to follow dietary pattern that is associated with lower risk of CRC. In light of this view, it is strongly recommended to follow diet rich in fruits, vegetables, fish, and poultry and whole-grain products. Figure 3.8 represents bioactive compounds present in plant against colon cancer.

3.12 Conclusion and Future Direction

In conclusion, epidemiologic evidence has shown a slight but substantial connotation between dietary intake and a diminution in CRC risk. We believe that CRC is largely preventable provided dietary pattern is well understood and applied in food choices. The higher incidence in more developed countries can be attributed, at least partially, to the Western lifestyle, with its high intake of red and processed meat, which has been reported to associate positively with higher risk of CRC. The global cancer reports published by the World Cancer Research Fund (WCRF) and the American Institute for Cancer Research (AICR) in 2007 and updated in 2011 listed red and processed meat as "convincing" factors that increase the risk of CRC. Many other dietary factors, such as fiber, fruit, and vegetables, may associate inversely with CRC risk.

Going forward, taking into account biological mechanisms consequential from experimental studies linking these factors with CRC, there is a convincing rationale to endure investigation into dietary strategies for CRC prevention. Technological advances in the form of novel experimental models and methodologic tools like metabolomics and microbial metaomics will indubitably yield mechanistic and fundamental insights that can be used to inform public health recommendations and restrain CRC incidence and mortality.

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Chapter 4
Recent Therapeutic Strategies for the Treatment of Colon Cancer

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Abstract Colon cancer is the most common cause of death and ranks second after lung cancer among the different cancer-related deaths. The treatment of colon cancer depends on many factors, including the nature of colon cancer (sporadic or hereditary), molecular factors, age, and stages of colon cancer. The current treatment approaches involve surgery/polypectomy, chemotherapy, radiotherapy, combination therapy, and immunotherapy, while advanced methods include gene therapy, cellular therapy, and targeted immunotherapy with concern to colon cancer, which is in a developing phase. However, none of the treatment options is devoid of side effects, and each of the therapeutics has drawbacks such as relapse of the tumor, lack of specificity in the targets, and resistance of the anticancer drugs. Therefore, advancement in the treatment approaches for colon cancer is highly needed. Though up to the next level, scientific technology is continuously working to develop better treatment for colon cancer. The advanced approaches focus on the specificity of the targets which can differentiate between cancerous cells and normal cells, delivery of the therapeutic agents by utilizing nanoparticles, viruses, and different types of encapsulation of drug molecules, while immunotherapeutic approaches involve monoclonal antibodies, cytokine treatment, cancer vaccine, and cellular therapies. A cancer vaccine is a new highlighting approach toward colon cancer in which common molecular defects of the cancerous cells are targeted. This chapter focuses on the different types of colon cancer, molecular factors, diagnostic targets, and treatment strategies for colon cancer.

Keywords Sporadic colon cancer · Hereditary colon cancer · Cancer vaccine · Nutritional therapy

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Abbreviation

5-FU	5-Fluorouracil
AJCC	American Joint Committee on Cancer
APC	Adenomatous polyposis coli
CC	Colon cancer
CEA	Carcinoembryonic antigen
CIMP	CpG island methylator phenotype
CIN	Chromosomal instability pathway
CRC	Colorectal cancer
CSCs	Cancer stem cells
EBRT	External beam radiation therapy
HCRC	Hereditary colorectal cancer
HNPCC	Hereditary non-polyposis colorectal cancer
HPCC	Hereditary polyposis colorectal cancer
MSI	Microsatellite instability pathway
SIRT	Selective internal radiation therapy
TNM	Tumors-nodes-metastases

4.1 Introduction

Colon cancer is one of the most severe causes of death among all cancers. The birthplace of this cancer is the large intestine, which covers the colon and rectum; thus, it also is known as colorectal cancer (CRC), rectal cancer, or bowel cancer. According to the reports of WHO (2019), 1.80 million cases of CRC are reported, which ranks second (first lung cancer) in the cause of death out of all the cancers. The primary symptoms of this cancer include weight loss, constipation, rectal bleeding, and alterations in bowel habits, abdominal anxiety, and weakness (Kim 2017). The key factors that cause CRC are lifestyle, older age (older than 50), obesity, other intestinal inflammatory diseases (i.e., Crohn's disease, ulcers, and bacterial infections), smoking, and genetic risk factors (very less) (Qureshi et al. 2018). Some dietary factors can cause CRC, such as excessive consumption of alcohol and less intake of vegetables, red meat, and processed meat (Gallicchio et al. 2008). Type 2 diabetes and microbial infection with specific bacterial species, i.e., *Bacteroides fragilis*, *Fusobacterium nucleatum*, and *Helicobacter pylori* (Strofilas et al. 2012), may increase the risk of CRC (Nakatsu et al. 2015; Henrikson et al. 2015; Kostic et al. 2013). However, increased physical activity and intake of dietary fibers, grains, vitamins (C and D), tree nuts, and fish may reduce the risk of CRC.

The epithelial cells which line the gastrointestinal tract are the main origin of the colon cancer that starts from a neoplastic precursor lesion (Ionov et al. 1993). When these epithelial cells divide more rapidly and lose the regulation and signaling control of the cell cycle, they converted into small polyps (small clusters of cells). At

early stages, these polyps are not harmful, but after 10–15 years, it may turn into the lethal stages of colon cancer (Medema 2013; Varnat et al. 2009). There are many influences behind the imbalance of cell cycle, such as alteration of genetic and epigenetic factors that deactivate various apoptotic genes and tumor suppressor genes and activate proto-oncogenes to convert into oncogenes. Altogether two major pathways merge all the genetic alterations during the accumulation of different mutations in the colon cancer. The first one is the traditional adenoma-carcinoma pathway (70–90%), and the second is the serrated neoplasia pathway (10–20%) (Nguyen et al. 2020). The classical adenoma-carcinoma pathway (chromosomal instability pathway (CIN)) initiates with the mutational changes in the Wnt signaling pathway, which mainly involve APC protein (adenomatous polyposis coli) (Ionov et al. 1993). This APC protein negatively regulates β-catenin protein, so when APC is mutated, it leads to the increase of the β-catenin in the cell that will translocate inside the nucleus and binds to DNA which will activate proto-oncogenes (Markowitz and Bertagnolli 2009a). The Wnt signaling defective pathways also cause mutations in the tumor suppressor gene p53 and apoptotic genes, i.e., BAX (Markowitz and Bertagnolli 2009b). The serrated neoplasia pathway involves the epigenetic changes by methylation of CpG islands; both hypo- and hypermethylations (specific position) are associated with the colon cancer (Markowitz and Bertagnolli 2009a; Kim et al. 2011).

The stages of the colon cancer are categorized by the TNM system (tumors-nodes-metastases), which defines the spreading of tumors from initial stages to metastasis in distant organs. This TNM system, which was discovered by the American Joint Committee on Cancer (AJCC), recognizes the depth of the invasion of the intestinal wall (T), the involvement of the lymphatic nodes (N), and the degree of metastasis in distant organs (M) (Dienstmann et al. 2017). Generally, the stages of cancer are categorized into I, II, III, and IV based on TNM value, which indicates the severity of colon cancer. There is also a difference between the left and right sides of the colon; here the location of cancer plays an important role in the metastasis of the CRC. The right-sided lesions in the colon follow the sessile serrated pathway, which mostly involves mucinous tumors, and the prognosis of this side is poor as compared to the left-sided lesions. However, the left-sided lesions of the colon follow the traditional adenoma-carcinoma pathway, but the prognosis of this side is better as compared to the right-sided lesions (Henrikson et al. 2015; Loree et al. 2018). Diagnosis of colon cancer is made by different screening analyses, depending upon the symptomatic and non-symptomatic conditions of the patients. The different stages of colon cancer diagnosed by colonoscopy, blood tests involve the level of CEA (carcinoembryonic antigen), CT scan of the gastrointestinal region, histopathology, and immunochemistry analysis of tumors (Di Como et al. 2015). The treatment strategies for colon cancer are determined by some key factors such as different stages of the tumor (early or late phases) and health conditions of the person (if having any other diseases). Early stages of this cancer can be cured by surgery. At the same time, chemotherapy, radiation therapy, immunotherapy, and combinations of different treatments may be useful in the later stages of colon cancer (Syn et al. 2017; Shaib et al. 2013).

4.2 Molecular Aspects of Colon Cancer

To identify each root of the colon cancer, we have to know about the different molecular factors of colon cancer (CC), which are associated with the progression of the tumor and also with their early diagnosis and prognosis. Generally, our body is adapted to a regular mutation rate, which is ~2.5 × 108 mutations/nucleotide/generation (Roach et al. 2010). If this rate is going to be abnormal, then the chances of the cancers increase due to the accumulation of different harmful mutations to the healthy cells and initiate its conversion into cancerous cells, which further leads to the expansion of the tumors. With regard to the colon cancer, there are many molecular aspects that have been studied, i.e., the traditional adenoma-carcinoma pathway/CIN (80–90%), serrated neoplasia pathway (30–45% of all CC), and microsatellite instability pathway (MSI) (10–20% in sporadic colon cancer and >95% in hereditary colon cancer) (Tejpar and Van Cutsem 2002). These pathways are mainly associated with sporadic disease (nonhereditary); apart from this, 20–30% cases of the CC are due to previous familial history (Jansen et al. 2020).

The mutations in various tumor suppressor genes, loss or gain of different parts of the chromosomes, and loss of heterozygosity are associated with the chromosomal instability pathway (CIN). This pathway involves the overexpression of proto-oncogenes such as BRaf [serine/threonine kinase (BRAF)] and KRAS [GTPase (KRAS)] and deletion of tumor suppressor genes, i.e., tumor protein p53 (TP53), APC, and SMAD4 proteins (Pino and Chung 2010; Lengauer et al. 1997). The series of mutations start with the APC protein, which disrupts the Wnt signaling, further leading to the mutations in T53, which is located on the chromosome 17 and increases the copy numbers of the KRAS gene (Fearon and Vogelstein 1990; Thiagalingam et al. 1996; Samuels et al. 2004). These different mutations result from the loss of different regions of the chromosomes, which include 1p, 5q, 8p, 17p, 18p, 18q, 20p, and 22q (Diep et al. 2006; Baudis 2007; Zarzour et al. 2015). These specific numbers of the chromosome designate the locations of the tumor suppressor genes such as APC on chromosome 5q, TP53 on chromosome 17p, DCC netrin-1 receptor (DCC), and SMAD family member (SMAD2 and SMAD4) on chromosome 18q. In comparison, the increase in the copy number of the chromosomal arms includes 1q, 8q, 12q, 13q, and 20q, which is primarily associated with the potential oncogenes (Willett et al. 2013).

The serrated neoplasia pathway [CpG island methylator phenotype (CIMP)] was assessed by epigenetic changes in the CpG islands of the promoter regions of genes. These epigenetic changes involve the aberrant DNA methylation at specific positions in the cytosine of the different promoter regions and lead to the silencing of the various tumor suppressor genes (Issa 2004). The status of the CIMP with varying stages of the tumor is utilized as early or a potential marker for diagnosis and treatment (Toyota et al. 1999). The profile of the tumors with CIMP+ or CIMP – concerning the involved mutations, i.e., CIMP+ tumors having a high rate of mutation in the BRAF and KRAS while CIMP – involve the high mutation frequency with the P53 (Samowitz et al. 2005; Ogino et al. 2006; Toyota et al. 2000; Shen et al. 2007).

Apart from sporadic colon cancer, 20–30% cases are genetically inherited, i.e., due to a family history of colorectal cancer with germ-line mutations and epigenetic effects on different genes. Hereditary colorectal cancer (HCRC) consists of a group of diseases and syndromes, which can be studied based on different molecular aspects and are divided into two large subgroups: hereditary non-polyposis colorectal cancer (HNPCC/Lynch syndrome) and hereditary polyposis colorectal cancer (HPCC)/familial adenomatous polyposis (FAP) (Pabón and Babiker 2019). Lynch syndrome is the most common type of hereditary CC, which happens due to defects in DNA repair machinery (mismatch repair (MMR) system). The panel of various genetic fluctuations in this syndrome is characterized by a microsatellite instability pathway (MSI) (Lynch and De La Chapelle 2003). Microsatellites are tandem repeats of 1–6 base-pair of DNA sequences. The replication errors in these repeats with the insertion or deletion of nucleotides, which generate abnormal alleles as compared to the wild type in the same individual (Porkka et al. 2019). Predominantly, the germ-line or somatic defects in the MLH1, MSH2, and MSH6 genes (genes associated with MMR) increase the lifetime risk of HNPCC (Boland et al. 2008). The HPCC is due to the germ-line mutations in the APC gene and covers 3–5% of all CC cases (Fig. 4.1) (Pabón and Babiker 2019; Cruz-Correa et al. 2017).

4.3 Diagnostic and Therapeutic Targets of Colorectal Cancer

Nowadays, noninvasive and early detectable biomarkers are needed to diagnose any diseases at the primary stage for better treatment. MicroRNAs are utilizing as a new diagnostic and therapeutic tools to detect the different stages of many cancers. miRNAs are the small (~22 nucleotides) noncoding circulatory RNA that regulate multiple genes by binding with 3′UTRs of the mRNAs (Bartel 2018). miRNAs regulate the different molecular mechanisms of the inflammation, invasion, and tumor progression in colon cancer (Nagaraju et al. 2016). Many miRNAs are reported that play a role in tumor suppression and progression of colon cancer, such as miRNA-143, miRNA-100, miRNA-126, miRNA-20b, miRNA-455, miRNA-139-3p, miRNA-1246, miRNA-182, miRNA-92a, and miRNA-214 (Nagaraju et al. 2016; Zhou et al. 2015; Yamada et al. 2014; Li et al. 2013; Akao et al. 2006). These miRNAs are categorized into two subgroups based on their expression level, which defines the nature of the tumor suppressor or oncogenes. miRNAs with tumor suppressor nature are miRNA-143, miRNA-101, and miRNA-142-3p, which means that the expression of these miRNAs is decreased in colon cancer (Zhou et al. 2015; Akao et al. 2006) (Shen et al. 2013). miRNAs (oncogenic) that are highly expressed in colon cancer, which are also known as oncomirs, are Lgr5, miRNA-1246, miRNA-155, and miRNA-92a (Tili et al. 2011; Zhou et al. 2013) (Wu et al. 2013). Out of these all miRNAs, only selected miRNAs could be utilized as biomarkers by analyzing their expression levels on the early or late stages of the colon cancer.

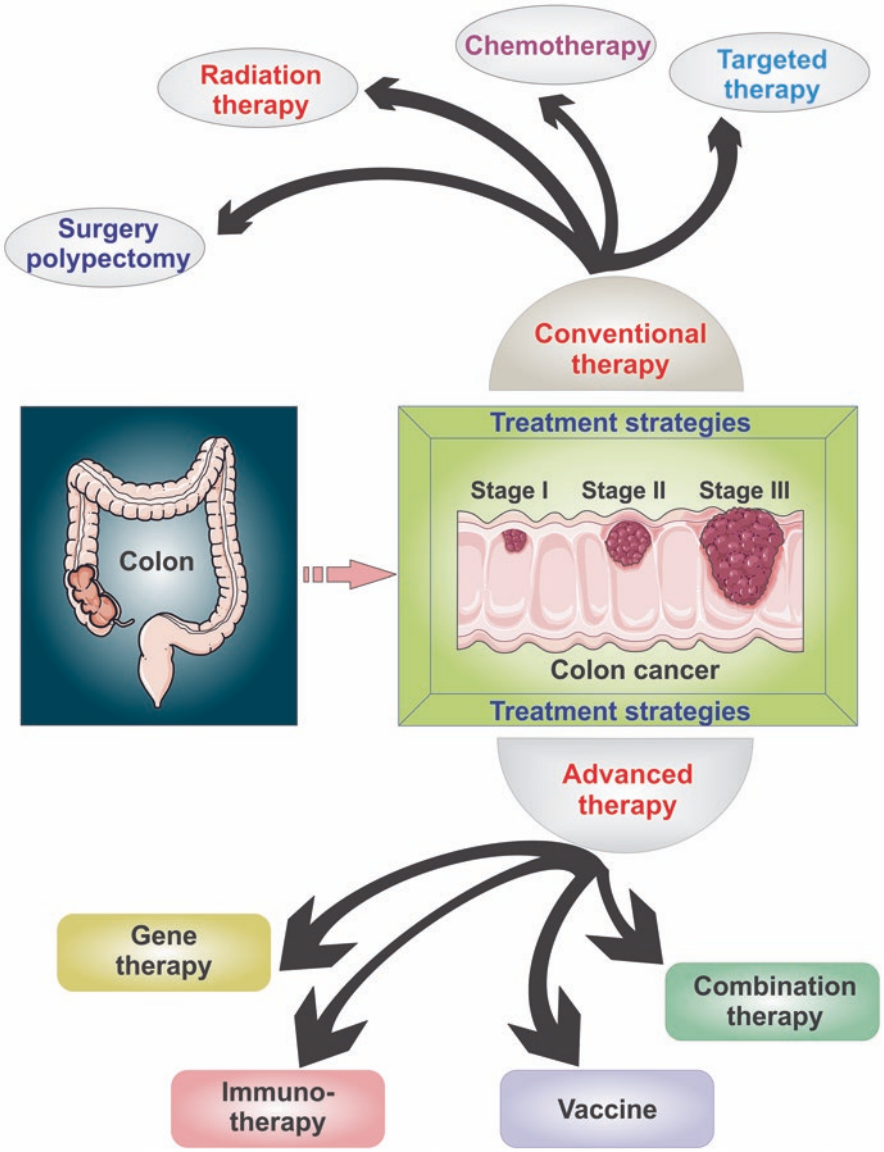

Fig. 4.1 Types of colon cancer with their molecular pathways

Overexpressed miRNAs could also be used as therapeutic targets by altering their expression level through gene silencing.

Apart from the miRNAs, Wnt signaling is also a reliable target for therapeutic purposes. Although it is very complicated to target the specific component of the Wnt signaling due to its association with other cellular signaling circuits (Anastas and Moon 2013), advanced and novel drug discovery approaches have tried to target

and successfully synthesize drug *celecoxib,* which is a COX2 inhibitor and directly inhibits β-catenin (CTNNB1)-induced transcription of proto-oncogenes (Smith et al. 2000; Tuynman et al. 2008). As the Wnt signaling is a central target for the treatment of the colon cancer, Wnt blocking antibodies and peptides are also utilized to reduce the proliferation of the tumor and induction of the apoptosis. The antibodies which neutralize WNT3A, FZD7, and FZD10 Wnt receptor proteins are used for the treatment of colon cancer (Anastas and Moon 2013) (Li et al. 2008; Pode-Shakked et al. 2011).

4.3.1 Treatment Approaches to Colon Cancer

The strategies used to treat colon cancer involve many conventional and advanced scientific methods. The selection of the type of the treatments depends on the stage of the tumor, the age of the patient, the immune system of the patient, and the lifestyle of the patient. With the attention of all these factors, different treatment trials display various kinds of positive or negative responses. The conventional methods for the treatment of the colon cancer involve (i) surgery/polypectomy, (ii) radiation therapy, (iii) chemotherapy, and (iv) targeted therapy. These methods are not enough for the complete treatment of the CC, so the scientists have tried to provide advanced alternative approaches to deal with the disadvantages of conventional methods. The advanced methods involve (i) immunotherapy/biotherapy, (ii) cancer vaccines, (iii) cellular therapies, (iv) gene therapies, (v) combination therapies, and (vi) nutritional supplement therapies (Fig. 4.2) (Mishra et al. 2013).

4.3.1.1 Conventional Methods

Surgery/Polypectomy

If CC is diagnosed at very early stages (I and II), surgery is an effective treatment for the removal of the polyp or tumor. However, some complicated surgeries are also done for the removal of late stage-specific localized incision. The very early stage cancer contained only in a polyp can be removed during the colonoscopy; this removal of the early polyp is known as polypectomy. Larger polyps can be removed by the small removal of the inner lining of the colon by using the procedure, namely, endoscopic mucosal resection. For more advanced polyps that cannot be removed by colonoscopy, nowadays, laparoscopic surgery is utilized to remove the small incision of the abdominal wall. Expert surgeons use instruments with attached cameras to analyze the video of the colon and remove the incision regions and can also take the samples of lymph nodes located near the polyps. If a large incision of the CC covers the small part of the colon, then it can be removed by partial colectomy in which the cancerous region and some parts of the healthy tissue are removed. After partial colectomy, the remaining part is reconnected with the colon or rectum,

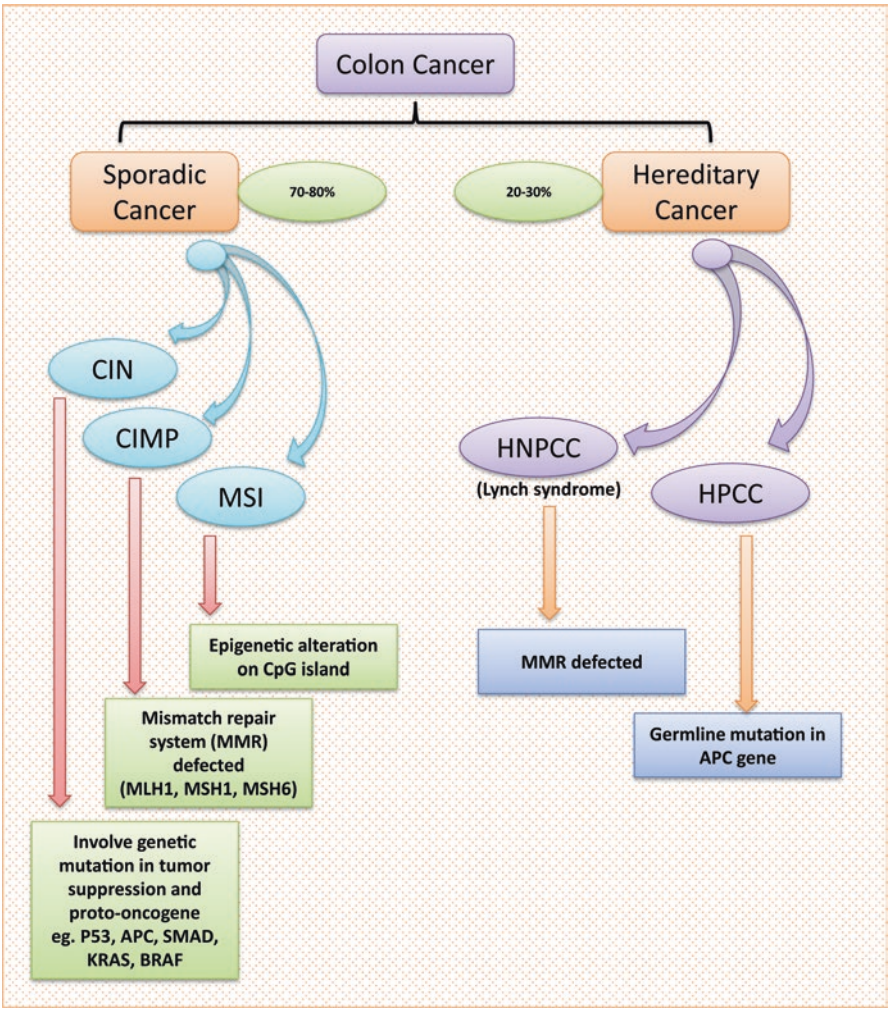

Fig. 4.2 Treatment strategies for colon cancer

but if it is not possible, then colostomy has to be performed for the removal of the waste of the body. The colostomy provides a stoma in the large intestine for the elimination of the stool (the alternative root of the rectum). It may be temporary to heal the surgery or may be permanent. Despite all these surgical treatments, there is a chance of reoccurrence of the tumor, which makes it again an incomplete treatment (Kristjansson et al. 2010; Taylor et al. 2002).

Radiation Therapy

Radiation therapy is useful to reduce the size of the tumor before the complicated surgery of tumor subtraction. The high-energy radiations (x-ray, radio waves, and protons) are subjected to the localized tumors via different roots of the body. The types of this therapy include external beam radiation therapy (EBRT), selective internal radiation therapy (SIRT)/brachytherapy, endocavitary radiation therapy, and interstitial brachytherapy. EBRT is provided by a machine outside the body with highly intense radiations. SIRT is mostly utilized in the case of CRC in which a radioactive source is inserted in the rectum near or inside the tumor. These radiations are not allowed to pass through the skin and other healthy tissues to reduce the side effects of the other organs. The endocavitary radiation therapies make use of a device with a small balloon inserted into the rectum via the anus and deliver high-intensity radiations. The main purpose of this therapy is to avoid complicated surgery and colostomy. The intestinal brachytherapy was subjected to a tube inserted into the rectum. The tube is filled with small bits of the radioactive matter, and radiations are allowed to a short distance for a short time to limit the harmful effects of the radiation. This method is used when the patient is not suitable for surgery. Radiation therapy is operational as adjuvant or additional therapy either pre- or post-surgery. The chances of reoccurrence are reduced in radiation therapy, but the numbers of side effects are still high such as skin irritation, nausea, rectal irritation, stool leakage, sexual problems, and fibrosis (Marshall 2008; Cupps et al. 1980).

Chemotherapy

Chemotherapy is the central treatment strategy for any cancer, which involves the utilization of various types of chemicals. The chemicals can be a plant alkaloid, antimetabolites, enzymes, alkylating agents, antitumor antibiotics, and hormones. Most of the time, chemotherapy is performed after the surgery if CC has been spread up to the lymph nodes, but it can also be used before surgery also reduce the size of the tumor for its easier removal. The main objective of chemotherapy is to destroy the cancerous cells by targeting the inhibition of the DNA replication, interfere with the chromosomal separation during the cell cycle, or directly provide a cytotoxic environment to the tumor. But the foremost disadvantage of chemotherapy is that it targets all rapidly dividing cells in the body, so healthy cells are also destroyed by this treatment. With the involvement of all rapidly dividing cells, only a small amount of the drug can reach the cancerous cells and again reduce the expected outcomes of the drug. Up to now, there is no single drug which can target only cancerous cells without affecting normal cells of the body. With regard to colon cancer, *5-fluorouracil* (inhibitor of thymidine synthase) has remained the first-line chemotherapeutic agent for treatment purposes (Poon et al. 1989; Gustavsson et al. 2015). Mostly, it is used with combinations of different drugs, e.g., leucovorin (a vitamin), capecitabine (Xeloda), oxaliplatin (Eloxatin), irinotecan (Camptosar), ramucirumab (Cyramza), cetuximab (Erbitux), aflibercept (Zaltrap), and panitumumab (Vectibix),

which act as adjuvants to improve the effectiveness of the drug (Wang et al. 2015). The drug regorafenib (Stivarga) is a new drug that can be used without 5-FU to treat CRC. Regorafenib targets the SHP-1-STAT3 axis; SHP-1 (SH2 domain-containing phosphatase-1) is a suppressor of CRC and inhibits the epithelial-mesenchymal transition (EMT) (Fan et al. 2016).

Targeted Therapy

To overcome the problems of conventional chemotherapy to target the cancerous cells only, novel drug delivery approaches have been introduced. The aims of novel approaches are to target only the cancerous cells, reduce the number of anticancer drugs, and have proper absorption and elimination of the drug. The advancement in the new approaches involves the utilization of nanoparticles (NPs), i.e., polymeric micelles, liposomes, nanocrystals, and dendrimers (Banerjee et al. 2017). The attention toward the NPs is due to its biocompatibility, small size, large surface area, nontoxic nature, and capability to conjugate with the various functional groups to target the specific cancerous cell (Wang et al. 2012). For the efficient and specific delivery of the anticancer drug to the tumor, NPs are conjugated with particular ligand or receptor (can include peptides, antibody fragments, and monoclonal antibodies), which overexpressed on the cancerous cells (Xie et al. 2016). Although 5-FU has been remained the first-line treatment of the CC, it has some drawbacks such as poor absorption, nontarget action on the healthy cells, and short half-life. To overcome these limitations of the 5-FU, researchers have created a pH-sensitive methacrylic-based copolymer for the covering of the drug to increase the half-life and absorption at the specific target (Ashwanikumar et al. 2012). Again to improve the circulation of the 5-FU in the blood, stealth liposome (covered with PEG (polyethylene glycol) with the functional moiety of PR_b (targeting ligand) (Garg et al. 2009). The liposome nanoparticles (LNPs) commonly used for the site-specific anticancer drug delivery and presently for the treatment of the CC, CPX-1 (irinotecan HCl: floxuridine), LE-SN38, and ThermoDox® LNPs, have cleared phase II clinical trials (Loira-Pastoriza et al. 2014; Patel 2008). For the more advancement in the drug delivery for the treatment of colon cancer, metal oxides, carbon nanotubes, and dendrimers are in the developmental phase (Esmaelbeygi et al. 2015).

4.3.1.2 Advanced Approaches to Treat Colon Cancer

Immunotherapy

Our immune system is a boon, to protect us against different diseases, but if it fails or its overprotection can be a curse. People with a healthy immune system can also be prone to develop cancer when it fails to distinguish between the normal and cancerous cells and recognize a cancer cell as normal cells. Sometimes the immune systems recognize the cancerous cells as foreign antigen but are unable to produce

an effective response to kill them, because these cells may secrete some immunosuppressive components (tumor-associated macrophages, myeloid-derived suppressor cells, and regulatory T cells residing in tumors and their products along with tumor-derived products) to downregulate the immune system (Morse et al. 2009). Scientists have tried to develop immunoregulatory medicine such as a ligand, antibody, and modified immune cells that can upregulate the immune system, destruct cancerous cells, and also reduce the side effects of other treatments. The advancement in immunotherapy is just like improving the performance of the body's natural immune system toward the treatment of cancer, and the complex functioning and regulation of the immune system provide more different strategies for cancer treatment. These strategies involve adoptive T-cell transfer (ACT), cytokine therapy, and administration of ligand and monoclonal antibodies. Mostly, the nature of immunotherapy is autologous, which means that specificity varies person to person and makes it costly (Halama et al. 2008). During the transfer of the modified T cell or any other immune cells, firstly, these cells and sample of the cancerous cells are harvested from the patient and activated toward the specific antigen (tumor) in vitro and placed back to the patient alone or with adjuvants (cytokine, i.e., IL-2). With regard to the immunotherapy, checkpoints PD-1 (programmed cell death protein 1) and CTLA-4 (cytotoxic T-lymphocyte-associated protein 4) are the central targets present on the T cells (CD4, CD8, dendritic cells, and mast cells), and their immunosuppressive ligands (PD-L1, PD-L2, CD-80, and CD-86, respectively) are present on the antigen-presenting cells, nonlymphoid tissues, and tumors (Seidel et al. 2018). Pembrolizumab (Keytruda) and nivolumab (Opdivo) are the checkpoint inhibitors that block the signaling of PD-1 and enhance the immune response of the T cells toward the killing of tumors. These inhibitory drugs are recommended to the patient having a high MSI stage of the CRC (Prasad and Kaestner 2017). Ipilimumab (Yervoy) is also an inhibitor of CTLA-4, utilized with the combination of the nivolumab to treat the advanced stages of the CRC (Sanghavi et al. 2020). Unfortunately, the immunotherapy is not much effective to the patient of colorectal cancer as compared to the other treatments, so more scientific efforts are needed to improve the outcomes of this therapy toward the CRC.

Cancer Vaccines

To boost our immune system and to fight against cancer, vaccines are developed as a new strategy. Vaccination is a form of active immunotherapy in which various immune cells are activated to deal with foreign antigens. The cancer vaccine differs from the vaccine of infectious diseases where infectious vaccines are given before the diseased condition, while the cancer vaccines are utilized after the occurrence of the diseases and autologous in nature. To enhance the immune response of a given vaccine, adjuvants are conjugated as a carrier or for the reduction in the dose of the vaccine. The effectiveness of any cancer vaccine depends on factors such as the development of host cell-mediated immunity, immune recognition of tumors, and effector mechanism and on the break tolerance of the immune system. The

challenge for a successful cancer vaccine is to discover an antigenic part of the tumor by which it can be designed and used to target the specific cancerous cells. The cancer vaccine may be antigen vaccine, vector-based vaccine, dendritic vaccine, and DNA vaccine. Though the approach of the cancer vaccination opens new doors for the treatment of the colon cancer, but it is also associated with some drawbacks which need scientific efforts for the improvements such as the autologous nature of the vaccine, conjugation of adjuvant with vaccine may increase the toxicity, and chemotherapy may alter the target of the antigen by mutation than that particular vaccine cannot work. With the subject of colorectal cancer, some vaccines are undergoing a different phase of the clinical trials. The OncoVAX is a patient-specific vaccine used in combination with BCG vaccine for stage II CRC patients who have cleared phase III clinical trials (Uyl-de Groot et al. 2005). The PolyPEPI1018 vaccine developed under phase ½ clinical trials is reported for the treatment of patient with metastatic phase of the CRC. This vaccine has six synthetic proteins to boost the immune response against the seven antigenic proteins commonly expressed in the CRC cells (Hubbard et al. 2019). The Ad5-GUCY2C-PADRE cancer vaccine has passed the phase I clinical trials with the patients of stages I and II (NCT01972737). GUCY2C (guanylate cyclase C) is a membrane receptor protein present on the intestinal epithelial cells and responsible for the synthesis of cGMP (cyclic guanylyl monophosphate). It is universally overexpressed in the CRC patient which makes it a suitable target for the immunotherapy. Adenovirus (Ad5) has been utilized for the delivery of this vaccine. The antigen-specific memory CD8+ T cells are induced after the administration of the vaccine, while any antibody response was not reported as the immune response. This vaccine provides a good efficacy with long duration and safety without any autoimmune toxicity (Snook et al. 2019).

Cellular Therapies

When specific modified immune cells are subject to fight with tumor cells, it may be studied as part of cellular therapies. The cells utilized for the treatment of different cancers include bone marrow mononuclear cell and mesenchymal stem cell. The immune cells such as dendritic cells, T cells (CAR T-cell therapy), and NK (natural killer) cells are taken from the patient and modified in the laboratory (in vitro) as per the requirement of the immune system to kill the specific cancerous cells and then transferred to the patient with the addition of IL-2 or IFN-γ. The chimeric antigen receptor (CAR) T-cell therapy in colon cancer has successfully implemented and got an effective outcome. The NKG2D CAR T cells with the combination of IL-2 and IFN-γ have shown antitumor effect and good cytotoxic activity in the human colorectal cancer cells, which provide a significant strategy against the CRC (Deng et al. 2019). NKG2D (natural killer group 2, member D) is a receptor protein expressed on the NK cells, CD8+ T cells, and γδ T cells. NKG2D ligands (NKG2DLs) are expressed in very fewer amounts but overexpressed in infected cells (cancerous cells). Enhancement of the expression of its receptors (NKG2D) on

the T cell increases the cytotoxic activity due to the binding of ligand and receptors (Antonangeli et al. 2017).

Gene Therapies

With the advancement in scientific technology, gene therapy has taken a better scope to overcome the drawbacks of conventional immunotherapies. The insertion of required genes in the host genome, such as genes associated with the expression of needed antibodies, cytokines, specific receptors, and ligands. The advantage of gene therapy is that once the specific gene is successfully transferred and inserted at the targeted place, it produces continuous production of the therapeutic molecule in the patient (Vigna and Comoglio 2015). The selection of the particular delivery system of the gene and targeting the specific cell or organ for the insertion is a typical challenge in gene therapy. The delivery system of the genes may involve viral or nonviral methods. With regard to cancer, genes associated with the inhibition of the angiogenesis and cell proliferation and therapeutic antibody-producing genes may be recognized as the potential targets of gene therapy. The recent advancements emerge with the utilization of antisense genetic tools, i.e., siRNA, miRNA, and CRISPR CAS9. For example, telomerase is an enzyme that regulates the length of the telomeres. At the same time, its overexpression in the cancerous cell leads to the immortality of the cell due to the increased length of telomeres. So this enzyme may be targeted at the molecular level by designing its antisense siRNA, which can inhibit the transcription of the gene. Telomerase may be used as vaccine target because of its over-expression by cancer cell that overexpresses the telomerase enzyme can be targeted by the vaccine (Byrne et al. 2008). The Wnt signaling components in CRC can be targeted by siRNA and miRNA to block the overexpression of β-catenin (He et al. 2005).

Combination Therapies

Different types of treatment strategies have different side effects, so to recover these side effects, combination therapy is an effective option primarily in cancer treatments. Chemotherapy itself is a combination therapy in which two or three drugs are used in combination as described above. Chemotherapy is the central therapy and is combined mostly with all types of therapies for an effective response. For example, chemotherapy and radiation therapies are combined with surgery to reduce the size of the tumor, while hormone therapy is also combined with surgery or with other types of therapies to accomplish the deficiency of essential hormones. The immunotherapy is combined with various cytokines, adjuvants, and immunostimulatory chemotherapeutic agents to enhance the immune response (Ménard et al. 2008). Apart from this, to target specific cancerous cells (to be distinguished from normal cells), different types of targeting agents are combined with central drug such as siRNA, nanoparticles, and monoclonal antibodies. For example, the drug

doxorubicin is combined with snail siRNA, which is encapsulated in the chitosan nanoparticles (ChNPs). The drug has antitumor activity, and the siRNA inhibits the overexpression of the mesenchymal transition gene in the metastatic phase (Sadreddini et al. 2017).

Nutritional Supplement Therapies

A diet with proper nutrition is the pre-most factor for a healthy life. If any imbalance occurs in the nutrition or unhealthy diet, it can make a home for different diseases, including cancer. Nowadays, many diseases are cured with different therapies in which a combination of nutritional therapy has taken a commonplace for the improvement in the treatment outcomes. The fermented wheat germ extract (FWGE) is used in combination with the anticancer drug dacarbazine that has shown increased antitumor activity by inhibiting the metastasis of cancer. The FWGE is associated with the induction of the apoptosis in cancer cells via a caspase-mediated pathway (Mueller and Voigt 2011). Chemotherapy has the chance of relapse of tumor after the treatment due to the presence of cancer stem cells (CSCs). Curcumin is reported for the inhibition of chemoresistant cells of CSCs, and its analog GO-Y030 inhibits the phosphorylation of the STAT3 in most of the cancer cells. So when dasatinib (Src kinase inhibitor) is combined with GO-Y030, it has reduced the risk of relapse of the tumor in the colon cancer (Nautiyal et al. 2011; Lin et al. 2011). The CRC patients with microsatellite instability have reported resistance against the drug 5-fluorouracil (5-FU). Quercetin is a plant flavonol; a study showed that it could induce the apoptosis via mitochondrial pathways and increase the expression of the p53 gene (tumor suppressor) in CRC cell lines. Therefore, to reduce the resistance of the 5-FU, it is incubated with quercetin, and the result has shown better outcomes as compared to 5-FU alone (Xavier et al. 2011). Healthy food can work as medicine for colon cancer, and garlic is one of them. An organo-sulfur compound diallyl disulfide is a potential component, which is used in the form of oil. It promotes the apoptotic pathway and reduces cell proliferation in the cancer cells, although its clear mechanism of action is not entirely clear (Altonsy and Andrews 2011).

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Chapter 5
Role of Bacteria in the Development of Cancer

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Abstract Cancer is a cellular state where normal growth and survival of the cells are altered by a series of genetic changes. These changes can be induced by different factors, internal as well as external. Microbial infections are one of the major external factors and estimated to be responsible for 20% of the human cancers. These are mainly caused by viruses, but other microbes like bacteria, molds, or helminths are also reported to play an important role. Colorectal cancers (CRCs) are the third most common type of cancers in terms of number of cases. Almost 10% of all new cancer cases belong to this category. The intestine is a home for a large number of bacteria, which forms one of the most intimate relationships with humans. While mostly this relationship is considered beneficial, yet it is naïve to think that it is always that way. Recent studies showed involvement of some intestinal microbiota in cancer formations in the colon. Important mechanisms of bacterial induction of cancers are modulation and evasion of immune response, induction of chronic inflammation, activation of specific signaling pathways, and production of carcinogenic toxins or effector proteins. This present review tries to summarize the available knowledge about the relationship between cancer induction and bacteria, with special emphasis on colon cancer.

Keywords Colorectal cancer · Microbiota · Inflammation · Signaling pathways

Abbreviations

AIEC	Adherent-invasive *Escherichia coli*
BFT	*Bacteroides fragilis* toxin
CDT	Cytolethal distending toxin

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DSBs Double-stranded DNA breaks
EPEC Enteropathogenic *Escherichia coli*
ETBF Enterotoxigenic *B. fragilis*
LPS Lipopolysaccharide
NTBF Non-toxigenic *B. fragilis*

5.1 Introduction

Cancer is a cellular state where normal growth and survival of the cells are altered by a series of genetic changes. These changes can be induced by different factors, internal as well as external. Internal factors are mainly genetic predisposition for the said changes and form the hereditary basis of cancer development. An important external factor for cancer development is interaction of the cells to the environment they are exposed to. Exposure to sunlight (Koh et al. 1996; Parkin et al. 2011), smoking and alcohol consumption (Blot et al. 1988; Siegel et al. 2015), and microbial infections (Khoury et al. 2013) are major culprits in this regard. Around 75% of skin cancers and head and neck cancers are attributed to sun exposure and tobacco consumption, respectively (Blot et al. 1988; Parkin et al. 2011). Similarly, the majority of cervical cancers and hepatocellular carcinomas are reported to be associated with viral infections such as human papillomavirus and hepatitis B and C viruses (Bosch et al. 1995; El-Serag 2012). Approximately 20% of all the cancer cases are reported to be caused by some type of microbial infections (Elsland and Neefjes 2018).

Peyton Rous (1910) was the first person to suspect the involvement of microbes in the development of cancers. His experiment involved transferring the material from a cancer tumor of a sick chicken to a healthy chicken and subsequent development of cancer in the healthy chicken. However, this work was almost forgotten as it was not reproducible in other organisms. Later in 1933, Richard Shope revived the interest in microbial cause of cancers after discovering a tumor caused by a virus in rabbits.

Currently, many cancers are reported to be caused by different viruses. However, exploitation of the mammalian host is not only done by viruses. Many bacteria, molds, and helminths survive inside the mammalian host cells by intensive interactions and exploitations of host cell mechanisms. Bacterial pathogens manipulate and exploit the host cell in various ways depending on the stages of their infection cycle. Such encounters modify the host cell signaling pathways and may result in the development of cancer. Many workers have reported a strong epidemiological link between cancers and various microbial infections (Table 5.1). Common examples include, but are not limited to, connections between *Helicobacter pylori* infections and gastric cancer (Kikuchi 2002), *Salmonella enteritidis* infections and colon cancer (Mughini-Gras et al. 2018), chronic *Salmonella typhi* infections and gallbladder cancer (Scanu et al. 2015), and *Schistosoma haematobium* infections and bladder cancer (Mostafa et al. 1999).

Table 5.1 A list of bacterial species involved in cancer development

Bacterial species	Type of cancer	References
Bacteroides fragilis (ETBF)	Colon cancer	Sears et al. (2015)
Chlamydia trachomatis	Cervical cancer	Koskela et al. (2000)
Chlamydia pneumoniae	Lung cancer	Zhan et al. (2011)
Enterococcus faecalis	Colon cancer	Balamurugan et al. (2008)
Escherichia coli (AIEC and EPEC)	Colorectal cancer	Swidsinski et al. (1998), Maddocks et al. (2009)
Fusobacterium nucleatum	Colon cancer	Castellarin et al. (2012), Holt et al. (2012)
Helicobacter pylori	Gastric cancer	Kikuchi (2002), Wang et al. (2014)
Salmonella typhi	Gallbladder cancer	Scanu et al. (2015)
Salmonella enterica	Colon cancer	Mughini-Gras et al. (2018)
Streptococcus gallolyticus	Colon cancer	Gupta et al. (2010); Butt et al. (2016)

Colorectal cancer (CRC) is the third most commonly diagnosed form of cancer worldwide, which accounts for about 10% of all new cancer cases (Can. Cancer Soc. 2017; Wong and Yu 2019). Mortality wise also, it ranks second among all cancers. Colon cancer alone is responsible for 850,000 new cases and a mortality of 550,000 individuals, in 2018 (Bray et al. 2018). The numbers may vary from country to country, showing lower rates in developed countries and higher rates in developing countries. These variations are likely due to increased screening and removal of precancerous polyps in developed countries, which is lacking in developing countries (Stewart and Wild 2014). If this trend continues, global increase in the cases of CRC is estimated to be 60% by 2030, which means over 2.2 million new cases and 1.1 million cancer deaths worldwide (Arnold et al. 2017; Torre et al. 2015).

Many environmental organisms come in contact with humans transiently, but the relationship of a man with his own microflora is the most intimate one. A human host contains millions of microbes in their body, and their relationship with these microorganisms is more or less permanent and unremitting. This symbiotic relationship is generally considered to be beneficial, which in most cases is true. Yet it is naïve to assume that this relationship is always the same and our continuous interaction with microbial flora is not causing any harm.

Colon is the most densely colonized organ which contains approximately 70% of the host's microorganisms (Gagniere et al. 2016). These microbes are involved in many functions inside the body. Animal studies involving germ-free animals have highlighted a possible role for the microbiota in various models of carcinogenesis (Vannucci et al. 2008; Lofgren et al. 2011; Dapito Dianne et al. 2012; Schwabe and Jobin 2013).

In this present review, different possible bacterial factors for bacteria-induced cancer formation are discussed along with a detailed account of some bacterial species associated directly with colon cancer development.

5.2 Bacterial Factors Involved in Cancer Development

Interactions between the host and its constituent microbiome are very diverse. Since microbes' behavior changes according to the host, their exact contributions to cancer development are hard to predict. Especially, this becomes more problematic in cases of pathogenic bacteria as they are reported to take advantage of the human host cell conditions in various ways depending on the stages of their infection cycle. Many mechanisms have been proposed by different workers for different bacteria in this regard. Induction of chronic inflammation and production of carcinogenic bacterial metabolites are some important bacterial mechanisms linked to cancer formation (Parsonnet 1995). The most specific example of the inflammatory mechanism of carcinogenesis is *Helicobacter pylori* infection. *H. pylori* is the first bacterium to be identified as a definite cause of cancer in humans by the International Agency for Research on Cancer (1994). To generate the inflammatory response in the host, many bacterial proteins and chemicals are used by these pathogens. Important ones are surface moieties, effector proteins and toxins, etc. These molecules interact with host cells and modify the essential host cell signaling pathways to cause cancers.

5.2.1 Cell Surface Components

Modifications of the bacterial outer surface in many pathogens are reported. It is important as the outer surface is the first bacterial cell component to directly contact the host cells. These surface modifications result in better bacterial survival within the host cell, as they help in host invasion and dodging the immune system of the host. This modification can be of many different types. One example is the development of a polysaccharide-rich capsule by Gram-negative bacteria, which shields deeper structures on the bacterial membranes to limit complement activation and prevent engulfment by professional phagocytes (Winkelstein and Tomasz 1978; Pluschke et al. 1983; Abeyta et al. 2003).

Some other bacterial pathogens modify their surface-exposed molecules to avoid immune recognition. *Helicobacter pylori* modifies both lipopolysaccharide (LPS) and flagellin that helps it to avoid recognition by host immune cells (Gewirtz et al. 2004; Tran et al. 2005). *Salmonella typhimurium* expresses enzymes (deacetylase and palmitoyltransferase) to modify lipid A, resulting in decreased lipid A-mediated activation of TLR4 and NF-κb (Kawasaki et al. 2004).

Intracellular pathogenic bacteria have surface proteins that promote host cell attachment and internalization. Examples include expression of surface adhesins by pathogenic *Neisseria* spp. that mediate selective interactions with certain cell types to exploit specialized host cell niches (Popp et al. 2001). Similarly, *Staphylococcus aureus* and *Borrelia burgdorferi* produce fibronectin-binding proteins to interact with host cells and stimulate bacterial engulfment by non-phagocytic cells (Raibaud et al. 2005; Meenan et al. 2007).

Along with cell invasion, bacterial surface molecules also manipulate host cell signaling cascades, which affects host cell integrity and ultimately can induce cancers. LPS is a major surface-exposed component of the Gram-negative bacteria, which is present in both pathogenic and commensal bacteria. It activates TLR4, resulting in activation of numerous downstream signaling pathways by TLR4-mediated signaling. Many inflammatory and immune responses, capable of promoting the development of adenomatous polyposis coli (APC)-dependent colorectal cancers, are the end result (Coleman and Haller 2017).

In *H. pylori*, a type IV pilus adhesin protein (CagL) controls a signaling cascade to upregulate gastrin secretion. Upon binding with β5-integrin, it manipulates integrin-linked kinase complexes and the downstream Raf, MEK, and ERK pathways (Wiedemann et al. 2012). This results in hypergastrinemia, which is considered to be a major risk factor for the development of gastric adenocarcinoma. Another outer surface protein of *H. pylori*, OipA, is responsible for EGFR activation and also for the stimulation of Akt and β-catenin signaling (Tabassam et al. 2009; Polk and Peek Jr. 2010).

The Fusobacterium adhesin A (FadA) of *Fusobacterium nucleatum* is another example of an outer surface protein playing a role in the development of cancers. Association between higher expressions of FadA and upregulation of Wnt signaling pathway genes, especially of oncogenic and inflammatory genes, is established in patients with colon cancer (Castellarin et al. 2012; Kostic et al. 2012). Details of this association are discussed in the next section.

5.2.2 Bacterial Toxins

Survival of the pathogenic bacteria in the host requires immune cell elimination along with the immune escape. The most used strategy employed by bacteria, for this purpose, is the secretion of cytolytic toxins. Genes for these toxins are generally located on pathogenicity islands, and specialized secretion systems are used for transport of these toxins out of bacterial cells (Costa et al. 2015). The best-studied example for toxin-mediated inhibition of host cell protein synthesis is diphtheria toxin, produced by *Corynebacterium diphtheriae* (Murphy 2011). These toxin-mediated assault strategies are primarily meant for creation of a favorable host cell environment, but toxins can also contribute to carcinogenesis mostly as a side effect. Toxin-mediated genomic instability and induction of cell death resistant cell signaling are the main mechanisms for cancer development (Rosadi et al. 2016).

The colibactin, Shiga toxin, cytolethal distending toxin (CDT), and endonucleases are the major protein toxins that are responsible for genome instability. These toxins induce host cell double-stranded DNA breaks (DSBs) and cause cell death.

Colibactin, secreted by *E. coli* strains, is a toxin related to the formation of DSBs and introduction of genomic instability in the host. These double-stranded DNA breaks by colibactin activate ATM, CHK1, and CHK2 pathways, which are primarily responsible for checking DNA damage in the cell. This leads to CDC25- and

CDK1-mediated G2- to M-phase cell cycle arrest and apoptotic cell death. Sometimes, the system does not work as intended which results in incomplete DNA repair and chromosomal instability, due to side effects of colibactin-induced mechanisms. These resulting phenotypes can promote cancer formation in the host cell (Nougayrede et al. 2006; Cuevas-Ramos et al. 2010).

Gram-negative bacteria like *E. coli*, *S. typhi*, *Shigella dysenteriae*, and *Campylobacter jejuni* produce CDT, which is made up of three subunits, CdtA, CdtB, and CdtC. Out of the three, CdtB is primarily responsible for double-stranded DNA breaks in host cells. However, sublethal doses of CDT can lead to accumulation of mutations in the genome, after prolonged exposure. It happens because the cellular system which detects DNA damage is affected by CDT. Survival of the toxin-exposed cells is ensured by MAPK upregulation by activation of NET1 and the GTPase RhoA (Guerra et al. 2004). As a consequence, genomic errors occur that underlie cancer formation.

Toxin-induced carcinogenesis can also occur by methods other than introduction of DSBs and genomic instability, which include inducing resistance to cell death signaling and promoting proliferative signaling. Some pathogenic organisms get benefitted from host cell survival, and toxins produced by them help host cells to survive. An example of such a toxin is *Bacteroides fragilis* toxin (BFT), which stimulates cell proliferation by cleaving the tumor suppressor protein E-cadherin (Sears 2009). Mechanisms involve the activation of the β-catenin/Wnt and NF-κb signaling pathways (Wu et al. 1998, 2004), similar to *F. nucleatum* FadA.

Other bacterial toxins involved in cancer formation include CagA and VacA proteins from *H. pylori* (Nakayama et al. 2009; Tabassam et al. 2009; Wang et al. 2014).

5.2.3 Bacterial Effector Proteins

Various intracellular bacterial pathogens, after host cell internalization, route across the endosomal system to end in the phagolysosome, a highly degradative organelle. During evolution of the pathogens, various mechanisms are evolved by intracellular bacterial pathogens to avoid phagolysosomal degradation. These mechanisms lead to either cytosolic growth of the pathogen or avoidance of phagolysosome formation.

Cytosolic pathogens like *Listeria monocytogenes*, *Shigella flexneri*, *Rickettsia* spp., and *Francisella* spp. grow in the cytosol of the host to avoid degradation (Fredlund and Enninga 2014). They produce effector proteins for this purpose that induce pore formation in the endolysosomal vacuole and ensure its subsequent rupture. For example, the listeriolysin-O protein, produced by *Listeria* spp., induces small membrane perforations and causes Ca^{2+} leakage from vacuoles. This increases the vacuolar pH, and subsequent vacuolar maturation is prevented (Henry et al. 2006; Shaughnessy et al. 2007).

Other pathogenic bacteria have been reported to hijack the phagosome to ensure a favorable replication niche. *Legionella pneumophila*, *Mycobacterium tuberculosis*, and *Salmonella* spp. are some of the examples, where production of effector

proteins ensures bacterial survival and replication within the phagosome (Tilney et al. 2001; Nagai et al. 2002; Rajashekar et al. 2014).

The use of effector proteins by intracellular pathogenic bacteria is primarily for their survival, by manipulating the host cell integrity. Sometimes, these manipulations lead to the development of a particular cancer type. Although no direct relationship is established, there are a lot of epidemiological data points in this direction. It is speculated that when effector proteins are introduced in the host cell, they disturb the cellular balance to cause cancers. Associations of *Salmonella typhi* with gallbladder carcinoma and *S. enteritidis* with colon cancer are such examples (Scanu et al. 2015; Mughini-Gras et al. 2018). Another *Salmonella* effector protein, AvrA, has been linked to colon cancer. A detailed mechanism of the AvrA contribution to cancer formation is discussed later in the paper.

5.2.4 Other Factors

Production of carcinogenic bacterial metabolites, which can cause mutations and are ultimately involved in cancer formation, is suspected to be one of the mechanisms in colon cancer formation. While the small intestine is the main organ for bile acid absorption, a small amount of bile acid enters the colon where it is reduced by different bacterial species. In vitro studies had shown the deconjugation of the 7α-hydroxyl groups from bile acids to cytotoxic 7α-dehydroxylating bile acids (deoxycholate and lithocholate) by bacteria present in the colon (Hirano et al. 1981). Promotion of cell proliferation and adenoma growth are reported by these chemicals (Stadler et al. 1988; Hill 1991), which in turn enhance carcinogenesis.

Another possible mechanism for cancer development through bacteria is by biofilm formation, biofilm being a structure produced by a community of bacteria. Biofilms could increase carcinogenic metabolites' concentration locally. Examples include increased concentration of polyamines, which in turn increases production of reactive oxygen species (ROS) (Stein et al. 2015). In addition, decreased expression of E-cadherin on colonic epithelial cells and an overactivation of IL-6 and Stat3 in epithelial cells are also associated with biofilms (Anders et al. 2014). All these mechanisms are reported to be involved in colon cancer.

5.3 Bacterial Species Involved in Colon Cancer Development

Association of different bacterial species has been shown with the development of gastrointestinal neoplasms, especially in the colon, rectum, and gallbladder. Important examples involve strains of *E. coli*, *Salmonella enterica*, *Fusobacterium nucleatum*, *Bacteroides fragilis*, *Streptococcus gallolyticus*, *Enterococcus faecalis*, etc. (Alhinai et al. 2019; Hernández-Luna et al. 2019).

5.3.1 Escherichia coli

Escherichia coli (*E. coli*) is a Gram-negative bacterium present ubiquitously in nature. Some of the pathogenic strains are associated with the development of colon cancer (Swidsinski et al. 1998), but the mechanism is not completely understood. They are known to promote chronic inflammation in the gastrointestinal tract, which may act as the trigger mechanism (Arthur et al. 2013). Another possibility is that the bacterium directly induces cancer formation by producing some effector molecules. Two such molecules cyclomodulin colibactin and effector protein EspF are produced and secreted by adherent-invasive *Escherichia coli* (Cougnoux et al. 2014) and enteropathogenic *Escherichia coli* (Pezet et al. 2013), respectively. These molecules are reported to have a role in the development and progression of colon cancer.

Adherent-invasive *Escherichia coli* (AIEC) is the most reported pathogenic *E. coli* strain in the case of colon cancer patients (Buc et al. 2013). It is well established that infection with AIEC stimulates IL-6 production (Lapaquette et al. 2012), which in turn induces the production of CEACAM6 (a cellular adhesion receptor associated with a carcinoembryonic antigen) (Kim et al. 2015). CEACAM6 acts as binding receptor for AIEC (Barnich et al. 2007) and facilitates the infection. After that, a secondary metabolite, colibactin, is secreted to induce colon cancer. Colibactin is produced by non-ribosomal peptide synthetase-polyketide synthase (NRPS-PKS) and has been associated with DNA damage (Vizcaino and Crawford 2015), by acting as an alkylating agent (Balskus 2015; Wilson et al. 2019). This induces DNA mutations and promotes tumor development.

Another pathogenic strain of *E. coli* commonly associated with colon cancer is enteropathogenic *Escherichia coli* or EPEC (Maddocks et al. 2009; Maddocks et al. 2013; Magdy et al. 2015). Stimulation of a metastasis-related cytokine, MIC-I, is shown after infection with EPEC in in vitro studies (Choi et al. 2013). The role of an effector protein, EspF, has also been established in cancer formation by EPEC. EGFR receptors in the host cell can be degraded via EspF (Garcia-Foncillas et al. 2014). A type III secretion system of EPEC is used to internalize EspF in the epithelial cells (Elliott et al. 2000). A lower level of DNA repair proteins MLH1 and MSH2 are also reported in the presence of EspF (Maddocks et al. 2009; Pezet et al. 2013). These DNA repair proteins are widely related to colon cancer (Pino and Chung 2011). EspF also promotes rupturing of tight junction proteins on the intestinal epithelium (like occludin and claudin), which in turn can promote detachment and dissemination of tumor cells. These events can also contribute to colon cancer metastasis (Peralta-Ramirez et al. 2008).

5.3.2 Salmonella enterica

Salmonella enterica is another common bacterium associated with colon cancer development (Mughini-Gras et al. 2018). Many serotypes of this bacterium are reported such as *S. typhi*, *S. paratyphi*, *S. enteritidis*, *S. typhimurium*, etc. (Spano

2016). It promotes carcinogenesis by modulating host cell immune response (Levine et al. 2015). It can induce chronic inflammation, which is responsible for DNA damage as well as increased proliferation and cell migration (Kuper et al. 2000).

Salmonella proteins associated with an increased risk of developing colon cancer are typhoid toxin and AvrA protein. Typhoid toxin increases cellular survival and disturbs the balance of microbes in the intestine, leading to intestinal dysbiosis (Belluz et al. 2016). These events help in the development of inflammatory bowel disease and ultimately of colon cancer (Kang and Martin 2017).

AvrA, an effector protein, is the main carcinogenesis-related protein of *Salmonella enterica*. A type III secretion system is used for secretion of AvrA to the outer surface (Ye et al. 2007). AvrA is mainly responsible for inflammatory and immune response dysregulation. Important mechanisms in this process are inhibition of IL-12, INF-γ, and TNF-α secretion (Lu et al. 2010), inhibition of the NF-κB signaling pathway (Liu et al. 2010b), inhibition of IL-6 transcription, and increase in IL-10 transcription (Lu et al. 2012). AvrA also induces cellular proliferation via activation of the Wnt/β-catenin pathway (Liu et al. 2010a), which is important in the signaling pathway associated with colon cancer development (Li et al. 2003). The JAK/STAT signaling pathway is also activated by AvrA (Lu et al. 2016), which regulates several mechanisms involved in carcinogenesis (Lu et al. 2017). Other than that, the function of the p53 transcription factor is affected by AvrA acetyltransferase activity (Wu et al. 2010), leading to cell cycle arrest and inhibition of apoptosis.

5.3.3 Fusobacterium nucleatum

Fusobacterium nucleatum (*F. nucleatum*) is a Gram-negative anaerobic bacterium that is adherent and invasive in nature. It is usually related to periodontal diseases and found in oral cavities (Han 2015). Studies showed the presence of this bacterium in different parts of the colon (El-Omar et al. 2008) and bacterial infections have been associated with colon cancer (Castellarin et al. 2012; Holt et al. 2012; McCoy et al. 2013). Even though the role of *F. nucleatum* in the development and progression of colon cancer is not very clear, different mechanisms have been proposed. The β-catenin signaling pathway is upregulated in colon cancer. Activation of this pathway in the presence of *F. nucleatum* is reported (Housseau et al. 2017). *F. nucleatum*-TLR4 interaction is primarily responsible for β-catenin phosphorylation and subsequent activation of the pathway (Chen et al. 2017). Similarly, *F. nucleatum* FadA-mediated activation of the Wnt/ β-catenin pathway promoting cell proliferation is also reported. Internalization of phosphorylated E-cadherin is achieved after binding of FadA to the extracellular domain. As a result, β-catenin is released and free to bind with transcription factors of the T-cell factor/lymphoid enhancer factor (TCF/LEF) family. Together, they control transcription of genes involved in cell proliferation, transformation, and apoptosis (Rubinstein et al. 2013). Additionally, a significant decrease on expression of the TOX family of proteins

after *F. nucleatum* infection has also been shown (Chen et al. 2018), which is associated with advanced tumors.

Another mechanism that is used by *F. nucleatum* for induction of colon cancer has been linked to inflammation. Patients who are suffering from colon cancer and also have *F. nucleatum* infection show an increased expression level for TNF-α and IL-10 in adenomas (McCoy et al. 2013) and an increased level of pro-inflammatory cytokines (IL-6 and IL-8) in tumors. These pro-inflammatory cytokines are regulated by the NF-κB transcription factor, which is found activated in colon cancer (Castellarin et al. 2012; Rubinstein et al. 2013). Increased levels of chemokine CCL20 are also reported upon infection with *F. nucleatum* (Ye et al. 2017). CCL20 is related to colon cancer progression (Petrosino and Rubie 2013) and TH17+ lymphocyte-mediated inflammatory response (Chin et al. 2015).

5.3.4 Bacteroides fragilis

Bacteroides are anaerobic, non-spore-forming, Gram-negative rods, which represent about 25–30% of intestinal microbiota (Wexler 2007; Arumugam et al. 2014). They play an important role in mucosal immune system development (Wexler 2007) and intestinal homeostasis (Xu and Gordon 2003). Among them, *Bacteroides fragilis* (*B. fragilis*) is most frequently isolated from clinical samples and is regarded as the most virulent *Bacteroides* species (Wexler 2007). Some *B. fragilis* strains have pathogenicity islands and produce *B. fragilis* toxin (BFT) or fragilysin (Pierce and Bernstein 2016). On the basis of the presence and absence of toxin, two *B. fragilis* strains have been described: (a) non-toxigenic *B. fragilis* (NTBF) and (b) enterotoxigenic *B. fragilis* (ETBF).

Both of these strains have different effects on colon cancer. In the presence of NTBF, a protection against colitis and colon cancer development is seen (Lee et al. 2018). On the other hand, ETBF has been associated with the development of colon cancer (Sears et al. 2015). A wide variety of clinical manifestations are reported in the presence of ETBF, ranging from a simple diarrhea to inflammatory bowel disease and colitis (Rhee et al. 2009).

Although the role of ETBF in the development of colon cancer is not clearly understood, different studies have shown that BFT has a major role in carcinogenesis induced by ETBF. This toxin is a multifunctional metalloproteinase, which could promote tumorigenesis. Activation of c-Myc (Platz et al. 2016) and subsequent overexpression of spermine oxidase (SMO) (Goodwin et al. 2013) are reported in the presence of toxin. SMO is an enzyme, which increases carcinogenesis-favoring reactive oxygen species (ROS). Another possible mechanism for cancer development is host immune system dysregulation by ETBF toxin. After bacterial infection, there is an induction in T_{reg} lymphocyte recruitment and accumulation in the intestinal lamina (Casero and Housseau 2016). This starts a cascade of downstream reactions, which triggers tumorigenesis. ETBF could also activate the β-catenin pathway, similar to *F. nucleatum*, which leads to carcinogenesis (Bengrine-Lefevre et al. 2011).

5.3.5 Streptococcus gallolyticus

Many observational studies as well as case control studies have established an association between *Streptococcus gallolyticus* infection and increased risk of colorectal neoplasia (Gupta et al. 2010; Butt et al. 2016). These associations were later confirmed experimentally in different mouse models (Kumar et al. 2017; Zhang et al. 2018). Mechanisms involved in cancer development are not yet fully elucidated. Abdulamir et al. (2009) reported an increase in expression of pro-inflammatory Nf-κB and IL-8, which may induce cell turnover and tumor. However, another observation suggested an increased level of nuclear β-catenin can drive cell proliferation in cultured colon cancer cell lines (HT29, HCT116, and LoVo), independent of inflammation (Kumar et al. 2017). Further, studies have also implicated a direct stimulation of epithelial cell replication by an unknown mechanism (Kumar et al. 2018).

5.3.6 Enterococcus faecalis

E. faecalis is closely related to *S. gallolyticus*; and similarly, it has also been shown associated with colorectal cancer (Balamurugan et al. 2008). Experiments with cultured epithelial cells tie the production of genotoxic peroxide and cancer development (Huycke et al. 2002). *E. faecalis* is also reported to influence cell cycle behavior and ultimately cause changes in the ploidy level of the cells (Wang et al. 2008). Changed ploidy levels can also be responsible for tumor formation.

5.4 Conclusions

It is more than a century when the relationship between cancers and microbial infections was first documented. Since then, many reports showing a possible link between different bacterial species and cancer development have been published. Microbial compositions as well as mechanisms involved vary depending upon the type of the cancer. Once this relationship is well studied, many different new ways will become available for the treatment of the cancers, which is the need of the time in light of the rising incidence of cancer cases. Although conventional therapies are still the backbone of cancer remedies, multiple studies had shown successful employment of bacterial species in various cancer treatments. The most important bacterial trait is the ability to specifically target cancerous cells.

Other than the causative and therapeutic role, bacteria can also play a role in early detection of the cancer. This aspect is again very important as early detection helps in better treatment.

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Chapter 6
Role of Bacterial Infection (*H. pylori*) in Colon Carcinogenesis and Therapeutic Approaches

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Abstract The Gram-negative (−) bacterium *Helicobacter pylori* colonizes in the mucus gel layer above the gastric epithelium and has been considered to be a major etiologic factor in several gastric health complications including peptic ulcers, gastric MALT, gastritis, and gastric cancer in approximately 50% of the world's population. Besides inducing colon carcinogenesis, the bacterium shows to be associated with different extragastric disorders. Eradication of *H. pylori* is reported to reduce 33–47% of gastric cancer; however, treatment of *H. pylori* against colon cancer is less effective in older people. Several targets of *H. pylori* to induce colorectal carcinogenesis have been hypothesized, and an association of gastric *H. pylori* with colorectal adenomatous polyps (CAPs) has been experimentally revealed. CAPs are precancerous lesions of epithelial cells and lead to colorectal cancer (CRC) from the adenomatous stage. Pathway analysis decrypts the cascade mechanism of CAPs via inducing matrix metalloproteinase (MMP),

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prostaglandin-endoperoxide synthase, and mucin with other molecular targets. Several therapeutic approaches have been established to eradicate *H. pylori* before inoculation and during histologic progression in early stages. This book chapter explains the impact and molecular mechanism of *H. pylori* in colon carcinogenesis and methods of therapeutic approaches.

Keywords *H. pylori* · Antibacterial drugs · *CagA* · Colon cancer · Colorectal adenomatous polyps

Abbreviations

AHR	Aryl hydrocarbon receptor
ARMS	Amplification refractory mutation system
BLI	Blue laser imaging
BMI	Body mass index
CA	α-Carbonic anhydrase
CAFs	Cancer-associated fibroblasts
CagA	Cytotoxin-associated gene A
CNN	Convolutional neural network
COX-2	Cyclooxygenase 2
CRC	Colorectal cancer
CT	Cholera toxin
CtsC	Cathepsin C
EMT	Epithelial-mesenchymal transition
FliD	Flagellar hook-associated protein 2
GI	Gastrointestinal
IL-8	Interleukin-8
JAK	Janus kinase
LCI	Linked color imaging
NBI	Narrow-band imaging
OMVs	Outer membrane vesicles
P-CAB	Potassium-competitive acid blocker
rFUVL	Fusion protein
RUT	Rapid urease test
SAT	Stool antigen test
T4SS	Type IV secretion system
TFRC	Transferrin receptor
UBT	Urea breath test
UreB	Urease B subunit
VIP	Vasoactive intestinal peptide
WOS	White opaque substance
YAP	Yes-associated protein

6.1 Introduction

Over a million cases with 783,000 deaths have been reported till 2018 of gastric cancer making it the fifth popular cancer (Venerito et al. 2019). A surveillance research article by the American Cancer Society reports facts and figures during 2020–2022 on colorectal cancer and shows that in 2020 there will be approximately 104,610 new cases of colon cancer and 43,340 cases of rectal cancer in the United States (US). It is also estimated that in 2020, 53,200 people will die from colorectal cancer (ACS 2020). Colorectal cancer (CRC) is characterized by a varied geographical distribution over the world due to diverse life habits and a higher mortality rate due to diagnosis at an advanced stage of the cancer.

Cancer is the uncontrolled growth of tissue, viz., cells, characterized by unchecked cellular division of abnormal cells. When this uncontrolled phenomenon occurs in the colon or rectum, it ends as colorectal cancer (CRC). The large intestine is composed of the colon, rectum, and anus, popularly called the large bowel; thus, CRC is also referred to as bowel cancer in the final segment of the gastrointestinal (GI) system. Anatomically, the colon is a muscular tube 1.5 m in length and 5 cm in diameter, divided into the ascending colon, transverse colon, descending colon, and sigmoid colon ending with the anus (15 cm). Due to different cellular origins of the colon and rectum, their cancers are classified separately despite showing anatomical proximity. Colon and rectal cancers have different molecular and clinical features with different associated risk factors (Lee et al. 2015; Burón Pust et al. 2017), e.g., physical inactivity results in increased susceptibility to colon cancer instead of rectal cancer. Tumors in the colon were also frequently observed in older people than younger people, black people than white, and females than males (Sineshaw et al. 2018; Siegel et al. 2017).

6.1.1 Colorectal Polyp

CRC originates from the epithelial cells lining the colon and rectum which has a higher rate, approximately 10^{10}, of replication per day. The relatively higher rate of replication makes it highly vulnerable to mutation and hence carcinogenesis. However, it does not apply to the small intestine. Accumulation of mutations in tumor suppressor genes as well as proto-oncogenes in epithelial cells lining the colon and rectum changes its morphology to a hyperproliferation of polyps, which are neoplastic growths. CRC commonly begins as a noncancerous growth in mucosal layers of the colon or rectum, known as a polyp. However, it is common in older people aged >50, especially women than men, only 10% of which leads to invasive or progressive cancer (Corley et al. 2013; Levine and Ahnen 2006; Risio 2010). The growth pattern of polyps classifies colorectal polyps into adenomatous (i.e., adenoma) and serrated polyps (toothed appearance under a microscope) (Øines et al. 2017). Based on biological features, serrated polyps are further classified into

sessile serrated polyps (SSPs), traditional serrated adenomas (TSAs), and hyperplastic polyps (HPs). Benign lesion in the colon or rectum as an adenomatous polyp has a strong potential to develop into cancer despite other pathways in CRC like ulcerative colitis (Wong and Harrison 2001) and hyperplastic polyps (O'Brien 2007; Jass 2003; Hawkins and Ward 2001).

The evolution of healthy colonic or rectal mucosa epithelial cells into life-threatening invasive carcinoma reflects several molecular and biochemical changes in adenomatous polyps and colorectal cancers. Mutation in the K-ras protooncogene, DNA hypomethylation, and allelic loss of tumor suppressor genes on chromosome 5 (5q21, adenomatous polyposis coli gene), chromosome 18 (18q, colorectal cancer gene), and chromosome 17p (p53 tumor suppressor gene) are various progressive multistep processes toward carcinogenesis in colon and rectal epithelial cells (Wong and Harrison 2001). Mutational activation of oncogenes with simultaneous loss of genes responsible for tumor suppression alters the biochemical mechanism which induces oncogene proliferation in the colonic mucosa leading to polyp formation and then to carcinoma.

6.1.2 Microflora

The human intestine is a nutrient-rich habitat for over 500 bacterial species allowing them to grow and reproduce, with the largest community in the colon region (Guarner 2006). The human intestine is continuously exposed to pathogenic as well as nonpathogenic microorganisms, but a symbiotic relationship between bacteria and the host protects sometimes the colon tissue against pathogens. Normally or incidentally, a breach in the microbe-mucosal barrier induces pathogenicity. The development of cancer reflects a cryptic interactive relationship among pathogens, the environment, and host factors (Guarner 2006). Anatomically, cardia-localized tumors differ epidemiologically when compared to tumors of the non-cardia gastric region. *Helicobacter pylori* infection is commonly seen in non-cardia gastric cancer and inversely seen in cardia cancer. Involvement of *H. pylori* in colorectal cancer surprisingly drew the attention of scientists and researchers across the world, and they found that *H. pylori* induces colorectal carcinogenesis through inducing a complex cascade mechanism.

6.1.3 Helicobacter pylori

Helicobacter pylori, also known as *Campylobacter pylori*, is a helix-shaped microaerophilic Gram-negative ulcer-causing bacterium (Fig. 6.1) that is thought to infect the human stomach via interaction with gastric epithelial cells, affecting nearly 50% of the world's population. Barry Marshall and Robin Warren first identified *H. pylori* in 1982 in a person suffering from gastritis and gastric ulcer (Warren and Marshall

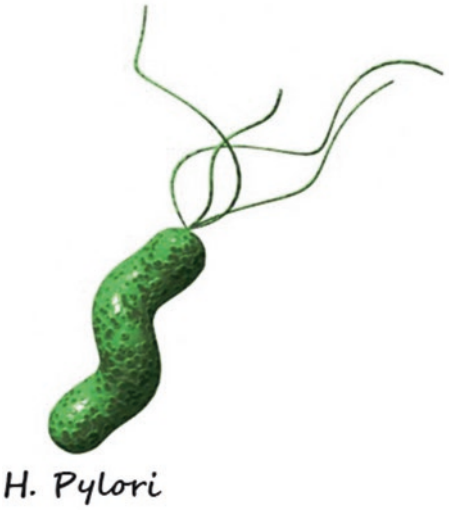

Fig. 6.1 Structure of *H. pylori*

1983; Marshall and Warren 1984). Infection of *H. pylori* commonly occurs at a young age evidenced through a *H. pylori* antibody test by various scientific communities in humans pooled from 17 populations in different geographical locations of the world (EUROGAST Study Group 1993). It has also been found that the infection scenario differs as the infection rate is nearing 90% in parts of Japan but only 33% in the United States (EUROGAST Study Group 1993). It has been proved that *H. pylori* infection has a strong positive correlation with lower socioeconomic status and dominantly occurs from person to person (Malaty 2007). Health complications due to *H. pylori* infection vary among different age groups with a higher gastrointestinal disorder potential in older people.

H. pylori has been reported to be present in mucosa-associated lymphoid tissue in the gastrointestinal tract (stomach, esophagus, colon, rectum) as well as specific tissue around the eyes (Bashir et al. 2013; Nocturne et al. 2019; Abbas et al. 2017). *H. pylori* is among the bacteria that can escape the extreme hostile acidic environment of the human stomach through producing the enzyme urease as well as placing themselves in gastric crypts of the stomach lining. The helical shape of *H. pylori* is thought to evolve in order to penetrate in the mucous lining of the stomach to protect themselves against the hostile acidic environment. Urease acts on urea which produces ammonia via hydrolysis which in turn neutralizes the acids near *H. pylori*. Letter on bacterium move to next region of gastrointestinal tract favorable environment (Brown 2000).

H. pylori is helix shaped, approximately 3μm in length and about 0.5μm in circumference with four to six flagella with flagellins A and B able to be identified under phase-contrast microscopy through various staining techniques (Gram stain, Giemsa stain, hematoxylin-eosin stain, Warthin-Starry silver stain, acridine orange stain) (Josenhans et al. 2000; Yamaoka 2008; Rust et al. 2008). *H. pylori* requires lower concentration of oxygen than that in the atmosphere and generates energy

through oxidizing the hydrogen molecule in the presence of its hydrogenase enzyme (Olson and Maier 2002). Besides hydrogenase, it also consists of various enzymes including oxidase, catalase, and urease. *H. pylori* consists of putative adhesins (largest protein family), iron transporters, porins, flagellum-associated proteins, and proteins with unknown function as a major outer membrane protein family (Kusters et al. 2006). Similarly to other Gram-negative bacteria, they have lipopolysaccharide and phospholipid molecular structures. Fucosylated O antigen mimics the gastric epithelium Lewis blood group antigens. Cholesterol glucosides were also present in the outer membrane of *H. pylori* (Kusters et al. 2006).

6.1.4 Classification

Domain: *Bacteria*
Phylum: *Proteobacteria*
Class: *Epsilonproteobacteria*
Order: *Campylobacterales*
Family: *Helicobacteraceae*
Genus: *Helicobacter*
Species: *H. pylori*

Many *H. pylori*-infected people are asymptomatic; however, other people exhibit duodenal, gastric, or colorectal problems. Chronic infection of *H. pylori* induces inflammation and gastric health complications including ulcer and cancer (Logan 1994; Parsonnet et al. 1991; Lochhead and El-Omar 2007). *H. pylori* infection has been reported to induce production of the cytotoxin-associated gene A (CagA) protein which activates an oncoprotein SHP2 with phosphatase activity. SHP2 deregulates the cell cycle leading to cancerous tissue (Lochhead and El-Omar 2007). With this ability, *H. pylori* has been classified a class I carcinogen by the International Agency for Research on Cancer (IARC) (IARC 1994).

Colorectal carcinoma has been significantly reported in infection with the CagA+ strain as compared to CagA- strain infection. It has been shown that not all strains of *H. pylori* induce carcinogenesis, and hence, combining non-virulent strains could dilute the virulent strain-mediated carcinogenesis. The CagA+ *H. pylori* strain causes inflammation and malignancy when compared to the CagA- strain (Beales et al. 1996; Maeda and Mentis 2007). Shmuely et al. show CagA's status as seropositive for *H. pylori* in 41 humans suffering from colorectal adenocarcinoma including research from 24 hospital-based controls reflecting CagA as seropositive for *H. pylori* (Shmuely et al. 2001).

Positive correlation between *H. pylori* and adenocarcinomas has been well reported in several epidemiologic research articles (Burnett-Hartman et al. 2008). Meta-analysis explains the relation of *H. pylori* and susceptibility toward colorectal neoplasia (Zumkeller et al. 2006). Surprisingly, *H. pylori* and colorectal neoplasia association does not mirror colorectal neoplasia's association with gastric cancer

over a geographic distribution especially in Japan where opposing trends have been recorded for these two cancers (Inoue and Tsugane 2005; Sung et al. 2005).

It has also been though that infection of *H. pylori* to colorectal tissue is not only the causative agent for colorectal cancer. Gastric *H. pylori* infection increases by-products. Gastrin has been reported to play a major role. Increased gastrin level (hypergastrinemia) in serum has been hypothesized to induce proliferation activity of the intestinal mucosa. Several researchers show a positive correlation with hypergastrinemia before diagnosis of colorectal neoplasia (Thorburn et al. 1998; Georgopoulos et al. 2006).

There have been several approaches to study hypergastrinemia and colorectal neoplasia including C-urea breath tests and polymerase chain reaction (PCR) methods to find their relationship with *H. pylori*. Several researches show mixed results. If *H. pylori* is a causative agent for colorectal cancer, it seems to be very cryptic, perhaps thought to induce via a pathogen virulence factor.

6.2 Pathogenesis: *Helicobacter pylori*

Global update till 2020 about *H. pylori* infection and pathogenesis explains its initial adaptation to and survival against an adverse acidic environment, colonization, biofilm formation, and interference in the metabolic pathway of the host cell with induction of neuroimmune cross-interaction leading to negative regulation of the gastric barrier till disease development.

6.2.1 Colonization and Chronicity

Initially *H. pylori* mimics the acidic condition of the stomach via dropping its cellular pH after infection. This lower cytosolic pH induces transcriptional factor HpNikR (nickel- responsive)-mediated expression of the ureA gene. Urease metabolizes urea and produces ammonia which neutralizes the acids near *H. pylori* (Jones et al. 2018). It is evidenced that urease knockout mutants fail to colonize, and hence restricting urease expression required for initial colonization as well as for chronicity (Debowski et al. 2017). Despite these, *H. pylori* reduces bicarbonate secretion from mucosal cells through regulating two transporters in duodenal cells: (1) solute-linked carrier 26 gene family A6 and (2) cystic fibrosis transmembrane conductance regulator. Lower availability of bicarbonate fails to protect the gastric barrier against strong acid and induces mucosal injuries and duodenal ulcer (Wen et al. 2018). It has been found that 8% of gene expressions of *H. pylori* were different in biofilms and planktonic. Several genes upregulated their expression in biofilms coding for envelop protein and flagellum construction to mitigate environmental stress. Biofilm formation induces the persistence of *H. pylori* in the stomach and hence chronicity (Hathroubi et al. 2018).

6.2.2 *Gastric Barrier Disruption*

H. pylori's presence has been recorded in the mucus, on the inner surface of the epithelium, as well as inside epithelial cells. It attached to the epithelial cells via producing adhesins against binding targets on gastric epithelia cells membrane preferentially with mucin 5 (MUC5AC) and Lewis (Le) determinants. It has been found that upregulated MUC5AC production and LeX and LeY determinant deposition in the gastric mucosa in *H. pylori* infection facilitate bacterium adherence. LeX and LeY in *H. pylori* also induce progressive colonization (Gonciarz et al. 2019). It has been shown that *H. pylori* penetrates deeply into the SPEM gland (spasmolytic polypeptide-expressing metaplasia gland) through interaction of SabA (sialic acid-binding adhesin) with sialyl-LX to avoid unfavorable environmental conditions (Sáenz et al. 2019).

Attachment of *H. pylori* to gastric epithelial cells is also regulated by the transferrin receptor (TFRC) through facilitating iron absorption (Hamedi Asl et al. 2019). Simultaneously, an overexpression of ferritin light chain (FTL) has been recorded in gastric mucosal cell predated by *H. pylori* which results epithelia cell differentiation into intestinal metaplasia (Hamedi Asl et al. 2019). Exposure of *H. pylori* to the gastric epithelial cell negatively correlates with the expression of aryl hydrocarbon receptor (AHR) and its suppressor (AHRR) evidenced in vivo and in vitro by siRNA silencing as well as immunochemistry (Reinfein et al. 2018). AHR and AHRR have a major role in antibacterial response, and their silencing increases the production of pro-inflammatory molecules and tumor necrosis factors in *H. pylori*-mediated gastric diseases.

Chronic infection of *H. pylori* has been found to induce vasoactive intestinal peptide (VIP) and a neuropeptide substance P (SP) in the gastric level and correlates with the potential of inflammatory response and severity of *H. pylori* colonization (Sticlaru et al. 2018). Bacterium-mediated gastric barrier disintegration induces inflammatory response and proliferation of gastric cells which in turn increases the genetic instability, hence causing progressive gastric cancer. Elimination of bacterium-mediated mutated epithelial cells and suppression of inflammatory response by apoptosis are beneficial to the host, but severe loss results in gastric barrier dysfunction. It has been found that *H. pylori* infection stimulates the invasion and metastasis of cancerous cells through positively modulating heparanase (HPA) expression responsible for cell migration and tissue remodeling (Liu et al. 2018c; Liu et al. 2018d).

6.2.3 *Colon Cancer: Pathogenesis of* H. pylori

Gastric cancer induced by *H. pylori* infection mediated through chronic inflammation in gastric mucosal cells has been reported meta-analytically to be a risk factor for colorectal cancer (Zhao et al. 2008; Zumkeller et al. 2006). There are various colorectal cancer markers elevated in *H. pylori*-mediated colon cancer like gastrin

hormone which acts as a gastric acid stimulant as well as mitogen (Sobhani et al. 1993). Colorectal and gastric cancers have some common etiology as colorectal cancer susceptibility increases in patients with gastric cancer and risk of primary gastric cancer increases in a person having colorectal cancer (Yoon et al. 2010). It has been also found the virulence potential and strain of *H. pylori* could induce gastric cancer as well as colorectal cancer. A *H. pylori* subtype with positive CagA-strains, serum antibodies to both CagA and VacA, and outer membrane vesicles has been reported in the pathogenesis of colorectal cancer.

6.2.3.1 Gastrin

Gastrin is a polypeptide hormone of 17 amino acids produced form G-cells located in the antrum, duodenum, and pancreas. Gastrin regulates the secretion of gastric acid while simultaneously regulating the pancreatic enzyme activity. Hypergastrinemia has been reported to be associated with colorectal malignancy through acting like a growth factor for the colonic epithelium (Creutzfeldt and Lamberts 1991; Sirinek et al. 1985; Chu et al. 1992). A person having Zollinger-Ellison syndrome shows an increased gastrin level with higher colonic proliferation (Sobhani et al. 1993).

The mechanism of gastrin in colorectal carcinogenesis is very complex; however, several researchers indicate that the gastrin level increases due to autocrine or paracrine secretion regulation by malignant intestinal cells (Hollande et al. 1997; Hoosein et al. 1990). Further analysis explains that gastrin upregulates the expression of cyclooxygenase 2 (COX-2, pro-inflammatory enzyme) and IL-8 inflammatory mediators. Inhibition of the expression of these inflammatory mediators has been reported to reduce colorectal cancer development (Chao and Hellmich 2010). Increased gastrin level increases the expression of COX-2 which stimulates the prostaglandin E2 level excessively leading to mucosal proliferation with reduced apoptosis and hence induced tumor growth. Recent studies show that not only gastrin but gastrin precursors could also be involved in colorectal cancer. It was found that *gastrin-gly* (gastrin precursor) upregulates the VEGF proangiogenic factor in colorectal cancer (Aly et al. 2004; Bertrand et al. 2010). A genetic bridge between colorectal cancer and hypergastrinemia was also thought about, as the k-ras oncogene induces gastrin expression, yet further scientific investigation is needed for safe conclusions.

The progastrin level increased significantly in CRC patients compared to CRA or healthy subjects. It has been linked that gastrin tropical action overexpressed its receptors, immunoreactive gastrin, COX-2 gene expression and Bax anti-apoptotic protein. This synchronously reduces the Bcl2 pro-apoptotic protein expression in colonic mucosa (Hartwich et al. 2001; Konturek et al. 2002). *H. pylori* induces colorectal cancer via gastrin and COX-2 through promoting proliferation and metastasis of gastric tumor cells (Raisch et al. 2015; Echizen et al. 2016). COX-2 promotes proliferation of cells, release of the angiopoietin, and endothelial cell apoptosis inhibition with activating matrix metalloproteinases (MMPs) in progressive tumor angiogenesis.

Persistence of *H. pylori* infection induces chronic gastritis reflecting reduced acid secretion which in turn produces high gastrin levels through negative feedback regulation (Konturek et al. 2000). People infected with *H. pylori* have been found to have increased basal and postprandial serum gastrin levels necessarily promoting colonic neoplasia. Patients infected by *H. pylori* with CRC are reported to have higher gastrin levels than CRC patients without *H. pylori* infection indicating the role of *H. pylori* in gastrin levels. It was found that increased gastrin level could be reversed after eradication of *H. pylori* infection (Penman et al. 1994).

6.2.3.2 Cytotoxin-Associated Antigen (CagA)

Cytotoxin-associated gene (CagA+) expressed by *H. pylori* is widely accepted to induce gastric cancer as well as colorectal cancer (Hartwich et al. 2001; Shmuely et al. 2001). Research on the CagA+ gene in gastric as well as colorectal cancer explains its role as a positive modulator of inflammatory stress in gastric mucosal cells when compared with the CagA- *H. pylori* gene. Its presence increases local and systemic inflammation leading to chronic atrophic gastritis and hence increases the gastrin level leading to colorectal carcinogenesis (Vanderstraeten et al. 1995; Limburg et al. 2002; Kim et al. 1999). Several researches explain that CagA+ seropositivity enhances inflammation status which in turn triggers the systemic cytokine cascade in premalignant lesion formation. It is also hypothesized that CagA+ strains exert endogenous carcinogen production through metabolism of ammonia products in the large intestine.

CagA+ promotes the persistence of *H. pylori* via impairing the neutrophil activation through downregulating cathepsin C (CtsC) via Src/ERK and Janus kinase (JAK) and activating STAT-3 pathways in the gastric mucosa (Liu et al. 2019).

An in vitro study of gastric epithelial cells by Li N et al. explains the CagA+ role in YAP (yes-associated protein)-mediated tumor suppression in Hippo. Li uses *H. pylori* wild-type cagA+ strains, PMSS1 cagA isogenic mutant strains, and recombinant cagA strain. They reported that the YAP pathway was induced by wild-type cagA+ strains and recombinant cagA, indicating susceptibility toward gastric cancer in the presence of cagA; however, cagA− mutants do not show effects on the YAP pathway (Li et al. 2018b). *H. pylori* CagA+ VacA+ strains modulate fibroblast differentiation into CAFs (cancer-associated fibroblasts) which in turn modulates normal gastric epithelial-mesenchymal transition (EMT) with enhanced invasiveness and migration activity (Krzysiek-Maczka et al. 2018).

In vivo researches on the gastric epithelium of humans and gerbils showed that the CagA strain induces proteomic changes in gastric cells through increasing the Rab/Ras signaling proteins (RABEP2 and G3BP2) and potency of malignant lesions (Noto et al. 2018). Type IV secretion system (T4SS)-mediated translocation mechanism of CagA+ into the host cell is cryptic; however, CECAMs (carcino-embryonic antigen-related cell adhesion molecules) are found to capture the

adhesin HopQ in the outer membrane of *H. pylori* and participate in CagA+ translocation (Noto et al. 2018). Neither β1-integrin nor any αβ-integrin on the cell surface has been observed in CagA translocation; but HopQ or CEACAM1, CEACAM5, and CEACAM6 deletion blocking the CagA translocation has been observed (Zhao et al. 2018).

Several results explaining null association of colorectal cancer risk with CagA might be due to different CagA isoforms, viz., strain of phylogeographic origin and its Glu-Pro-Ile-Tyr-Ala motifs (de Sablet et al. 2011; Hatakeyama 2009; Sicinschi et al. 2010). However, it further needs detailed prospective studies.

6.2.3.3 Vacuolating Cytotoxin A (VacA)

H. pylori with its eight different virulence constituents could be associated with gastric cancer and colorectal cancer, most notably CagA and VacA. Five different proteins of *H. pylori* have been reported in 60–80% of colorectal cancer. It has been evidenced through an in vivo study that VacA expression by *H. pylori* helps in the colonization into the host (Salama et al. 2001). Ammonia enhances the VacA toxicity potential, produced during the bacterial urease activity. Foegeding et al. reported that the presence of NH_4Cl induced the toxicity potential of VacA by increasing its intracellular stability in spite of altering the toxin during its trafficking through autophagosomes or lysosomes (Foegeding et al. 2019).

The amount of VacA antibody is reported to be maximum during the latter stage of colon cancer as well as early onset. The association of colorectal cancer and level of antibodies for VacA is thought to be related with gastrin hormone, mediated COX-2 protein expression, and their secretion (Kountouras et al. 2008). Administration of *H. pylori* with VacA significantly induces the inflammatory response and gastrinemia (Alvarez et al. 2006; Brzozowski et al. 2006; Konturek et al. 2003); however, this phenomena was reversed after eradication of *H. pylori* (Gatta et al. 2011). *H. pylori* VacA targets the mitochondria by creating pores in the membrane of host epithelial cells and then enters into the host cell cytoplasm via forming a vacuole. Multifunctional toxin VacA has several targets in the cytoplasm through which it blocks T-cell proliferation and induces apoptosis cascade by arresting the cell cycle (Palframan et al. 2012).

Several real-time PCR and histopathological examinations of the gene coding for cagA, vacA, and iceA were analyzed; and it was found that s1as1bm2, s1a1b, and s2m2 subtypes of VacA, respectively, induce 90.0%, 8.01%, and 84.2% of chronic gastritis in Saudi Arabia and the Southwest Asia region (Akeel et al. 2019). VacA signal and mid-region sequence phylogenetical analysis shows s1m1 as the most prevalent variant of *H. pylori* VacA causing gastro-duodenal disorder in Assam, India (Akeel et al. 2019). Mechanisms of *H. pylori* infection-induced colon cancer are given in Fig. 6.2.

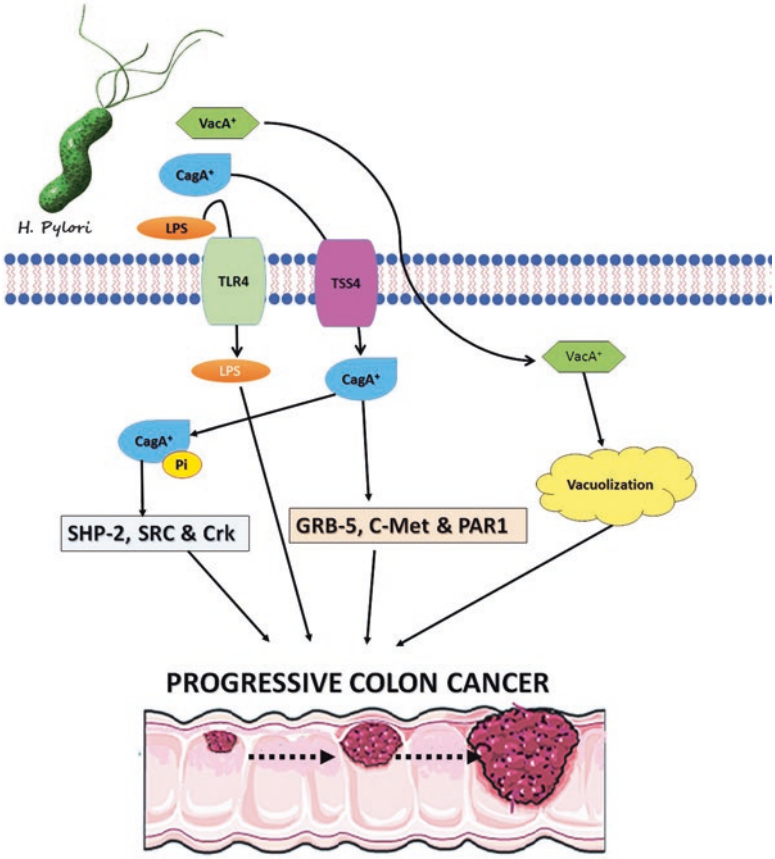

Fig. 6.2 Proposed mechanisms of colon cancer

6.2.3.4 Outer Membrane Vesicles (OMVs)

The active virulent component of *H. pylori* has been reported to exist in outer membrane vesicles (OMVs) which internalize into the gastric epithelium of host cells inducing the signaling pathway and mediate cellular apoptosis. It induces immunocompetent cell apoptosis and thus reduces immune responses helping in the disease development (Chmiela et al. 2018). Virulence factors are soluble in nature with cell surface bound or injectable ability to gastric epithelial cells via secretory system T4SS. Several researches are looking at OMVs produced by *H. pylori* in bacterial antigen distribution.

It has been found that OMVs' size explains their protein content, immunological potency, and entry into host epithelial cells. OMVs with 90–150 nm circumference enter through endocytosis and macropinocytosis; however, OMVs with reduced size, till 20–100 nm in circumference, enter through caveolin-dependent endocytosis (Turner et al. 2018). Ronci et al. found α-carbonic anhydrase (CA) in OMVs produced by various *H. pylori* strains (especially biofilm and planktonic phenotype) by using NMR and mass spectrometry. Their results explained that CA and urease play important roles in reducing acidity of gastric juice in *H. pylori* infection (Ronci et al. 2019).

6.2.4 *Colon Polyps and Metabolic Syndrome in* H. pylori *Infection*

Several biochemical parameters have been reported to affect colon polyp occurrence in patients infected by *H. pylori* including blood lipid profile, body mass index (BMI), and glucose level. Metabolic syndrome has also been reported as a risk factor of colon polyp occurrence. One study on 2244 patients explains that accumulation of visceral adipose tissue occurs in colorectal adenoma in a dose-dependent manner (Im et al. 2018). Similarly, hypertension and increased HbA1c with abdominal obesity make subjects highly susceptible to polyps (Fliss-Isakov et al. 2017).

Uric acid is another important biomarker and initiates strong pro-inflammatory effects (Kushiyama et al. 2016). Monosodium urate activates toll-like receptor and thus increases pro-inflammatory cytokines production level by leukocyte. Neoplasm formation in progressive cancer has been found to be regulated by pro-inflammatory factors; hence, serum uric acid is hypothesized in systemic inflammation and polyp cancer transformation (Dovell and Boffetta 2018).

6.3 Transmission of *Helicobacter pylori*

People from various professions (forestry, agriculture, fishery, sewage work, and mining) with not enough knowledge of *H. pylori* have been reported to have greater susceptibility to *H. pylori* infection. A Portuguese study explains health professionals working in these areas have a higher susceptibility to gastrointestinal health complications (Kheyre et al. 2018). A lower risk of *H. pylori* infection in the armed forces has been reported when compared to the general population. Another research reported a susceptibility of 64.3% for nannies, 43.2% for professional workers, 41.2% for industrial workers, and 35.2% for students in the United Arab Emirates (Khoder et al. 2019).

Spread of *H. pylori* through water has been reported by various scientists. In Peru, drinking of unboiled water and consumption of watercress have been reported as most causative factors for *H. pylori* infection in children in the 6–14 age group (Aguilar-Luis et al. 2018). No proper hand sanitization after toilet use has also been found as another reason for getting infected with *H. pylori* in Ethiopia (Melese et al. 2019). Tap water drinkers also are more susceptible than bottled water drinkers in the United Arab Emirates (Khoder et al. 2019).

Food could play an important role in the transmission of *H. pylori* according to socioeconomic status and dietary habits. Zamani et al. reported the presence of *H. pylori* in milk samples of cow and sheep, and thus they could also act as good reservoirs of *H. pylori* besides humans. It has been reported that *H. pylori* could survive in temperatures below 30 °C as well as pH in the range 4.9–6.0 in edible items like fresh fruit, milk, and vegetables. Milk containing urea could facilitate the prolonged survival of *H. pylori* (Zamani et al. 2017). Nature and intake style of food could possibly explain the transmission and infection of *H. Pylori* in human. Uptake of uncooked or mildly cooked non-vegetarian food items like mussels and various mollusks (sea foods), as well as raw vegetables like pepper, tomatoes, etc., significantly induces susceptibility to *H. pylori* infection. Consumption of meals from street vendors having poor hygiene has also a positive correlation (Monno et al. 2019). In spite of all these evidences, further research is required to find out correlation of *H. pylori* transmission with dietary habits.

Person-to-person transmission of *H. pylori* has been reviewed and evidenced by various researchers especially in family members (Kayali et al. 2018; Mladenova and Durazzo 2018). In Turkey and Ireland, a similar result is reported that *H. pylori* infection is more prominent in a larger family having an infected member (Palanduz et al. 2018; Dolan et al. 2018). Transmission was reported to be more frequent between siblings as well as children and their mothers (Rowland et al. 2018). Osaki et al. reported that gut microbiota could also help in *H. pylori* transmission (Osaki et al. 2018). Transmission is four times more likely from one sexual partner having reflux symptoms with *H. pylori* infection to the other partner (Sgambato et al. 2018).

6.4 Diagnosis of *Helicobacter pylori* Infection

Several methods like endoscopic imaging, immunohistochemistry, various molecular PCR methods, and biochemical analysis of the blood and stool are frequently used for analysis of *H. pylori* infection. However, analysis could be categorized in two major groups, viz., invasive (endoscopic imaging, histology, rapid urease test, and molecular methods) and noninvasive methods (urea breath test, serology, and stool antigen tests).

6.4.1 Endoscopic Imaging

There are several researches explaining the significance of *H. pylori* infection detection through specific endoscopic images. Endoscopic accuracy evaluation for *H. pylori* infection and status has been performed with mild atrophy in Japan evidencing the highest 89% nodularity and 77% mucosal swelling accuracy rates. Gastric black spots have also been observed through conventional imaging for knowing the presence or absence of *H. pylori* in eradication therapy. The detection pattern in endoscopic imaging could be (i) spotty pattern, (ii) molted pattern, or (iii) cracked pattern. The spotty pattern represents infection of *H. pylori*; however, molted and cracked patterns, respectively, represent intestinal metaplasia and post- inflammatory structural changes after eradication of *H. pylori* infection (Nishikawa et al. 2018).

Histological diagnosis through light endoscopy evolved through involvement of NBI (narrow-band imaging), LCI (linked color imaging), and BLI (blue laser imaging) from random biopsies to targeted biopsies in the stomach. A recent NBI-magnified endoscopic biomarker of *H. pylori* infection in intestinal metaplasia has been observed as a white opaque substance (WOS). WOS has been recorded in 28.4% of *H. pylori* patients, and its level reduced significantly to 3.2% after *H. pylori* eradication. Strong acidity inactivates the lipase enzyme and hence absorption of the lipids, reflecting the mechanism of WOS formation (Togo et al. 2019). More accurate NBI-magnified reddish depressed lesions have been significantly recorded in gastric cancer patients after eradication of *H. pylori* (Kotachi et al. 2018).

NBI uses blue light of 415 nm in magnifying endoscopy for vascular architecture imaging. This method is used in premalignant gastric cancer detection with 88% accuracy and shows superiority over serum pepsinogen I/II ratio methods with 74% accuracy (White et al. 2018). BLI uses two monochromatic lasers and is reported with 93% identification rate of early gastric cancer (EGC) as compared to 50% of white light imaging (Dohi et al. 2019). Another method capturing the real image with sufficient contrast known as LCI with magnifying endoscopy has also been performed for observing mucosal microstructure; however, its accuracy failed in improving the detection of *H. pylori* infection (Chen et al. 2018c).

Nowadays, artificial intelligence systems are being incorporated to improve the diagnosis of *H. pylori* infection and related disorders with LCI and BLI [9]. Another artificial intelligence-based algorithm using a dataset of a large number of images known as convolutional neural network (CNN) is used to diagnose *H. pylori* infection through diagnosing images as positive or negative (Shichijo et al. 2019).

6.4.2 Molecular Methods

Polymerase chain reaction with several modifications is frequently used to detect *H. pylori* infection and its resistance against clarithromycin and other macrolides. This technique has been performed in first-line eradication therapy to assess resis-

tance against various drugs during therapy, with a higher sensitivity and advantage over culture methods.

Iannone et al. reported the accuracy of real time PCR for stool sample in detection of *H. Pylori* over Urea Breath Test (UBT). They also used *H. pylori*-specific sequences to detect the mutation in a resistant bacterium against levofloxacin and clarithromycin (Iannone et al. 2018). Yu et al. introduced qRT- PCR for assessment of *H. pylori* infection using miRNAs as a biomarker in plasma. They reported the increased expression levels of four types of miRNAs in plasma of patients infected by *H. pylori* as compared to healthy subjects. They referred to these four miRNAs collectively as a "biomarker signature for *H. pylori* infection" (Yu et al. 2019).

Further advancement of PCR as droplet digital PCR (ddPCR) using thousands of oil droplets in small volume samples increases the sensitivity and detection efficiency for any genotype in low quantity in a mixed population. Sun et al. reported that ddPCR accurately detects the clarithromycin-resistant genotypes of *H. pylori*. They also found that ddPCR could accurately detect it in heteroresistance (Sun et al. 2018). Talarico et al. compare the ddPCR sensitivity to histology to determine *H. pylori* infection and clarithromycin resistance. Their finding showed the sensitivity of ddPCR as 93% in the detection of *H. pylori* infection (Talarico et al. 2018).

A study was performed by Bazin et al. to evaluate the comparative effect of PPI on test results of immunohistochemistry, histology, culture methods, and RT-PCR. They found that the PCR method was unaffected by the use of PPI as compared to other methods (Bazin et al. 2018). Lower sensitivity for *H. pylori* detection in humans (who had gone through eradication therapy previously) by the culture method has been reported due to migration of the bacterium to the antrum from the corpus after failing the eradication therapy. Bachir et al. used genotypic as well as phenotypic testing parameters for *H. pylori* resistance against tetracycline, clarithromycin, and fluoroquinolone by using molecular detection methods and antimicrobial susceptibility by culture methods (Bachir et al. 2018).

Another qRT-PCR method combined with the amplification refractory mutation system (ARMS-PCR) has been reported as fast and simple with higher specificity and sensitivity in the detection of *H. pylori* and/or its resistance against clarithromycin. ARMS-PCR is frequently used to detect the point mutation due to resistance against clarithromycin in patients who failed in *H. pylori* eradication therapy. A 98.7% similarity was recorded between ARMS-PCR and sequencing methods; however, similarity was 97.1% in ARMS-PCR and E test (Zhang et al. 2019).

6.4.3 Histology

Cresyl violet staining was used systematically to detect infection of *H. pylori* in gastric biopsies before 2014. However, after 2014, immunohistochemistry or cresyl violet staining produced similar results, i.e., visibility of *H. pylori* infection in only active cases of chronic gastritis (Benoit et al. 2018). El-Zimaïty et al. show that in histology it is necessary to ensure the presence of the oxyntic mucosa as well as the antral mucosa to eliminate the false-negative result (El-Zimaity et al. 2018).

6.4.4 Rapid Urease Test (RUT)

A comparative study of over 150 patients in Germany by RUT and histology explained that RUT is more sensitive in diagnosing infection of *H. pylori* in gastric biopsies. RUT shows another advantage of molecular detection of resistance against clarithromycin by *H. pylori* bacteria (Dechant et al. 2019; Baroni et al. 2018).

6.4.5 Urea Breath Test (UBT)

Urea breath test (UBT) is based on urease-mediated hydrolysis of urea which in turn releases CO_2 into the blood and to the lungs. Generally, 13C-labeled urea is used orally in tests; however, 14C-UBT could also be used. Released carbon isotope in breath could be measured by using infrared spectrometry and/or isotope ratio mass spectrometry.

A study by Eisdorfer et al. reported that UBT values significantly vary by age, sex, socioeconomic status, lifestyle, and body mass index (BMI). They found that UBT values were high in old patients vs. young patients, women vs. men, patients with low socioeconomic status, and nonsmokers as compared to smokers (Eisdorfer et al. 2018). A similar result was also reported by Perets et al. using UBT on over 235,000 patients (Perets et al. 2019). Coelho et al. reported that the 13C urea substrate locally synthesized in Brazil expresses similar performance in diagnosing infection of *H. pylori* (Coelho et al. 2018).

A comparative diagnostic study of frequently used noninvasive tests, viz., UBT, serology, and SAT (stool antigen test), in *H. pylori* diagnosis concluded that in 99 studies with 90% specificity, the diagnostic accuracies were 93%, 92%, 84%, and 83%, respectively, for 13C-UBT, 14 C-UBT, serology, and SAT. For this study, a standard reference of histopathologic results was taken. Authors proposed that hierarchy of these tests needs further confirmatory results globally (Best et al. 2018).

6.4.6 Serology

It has been found that monoclonal SAT and UBT diagnostic methods are better than serology in acute *H. pylori* infection, but still serology could be a diagnostic method of choice in patients with gastric atrophy, bleeding ulcers, gastric carcinoma, and mucosa-associated lymphoid tissue lymphoma and patients taking PPIs or antibiotics. Three antigens, CagA, Tip-α, and HP0175, are used to identify current and past infections of *H. pylori* and atrophic gastritis. The presence of one or more antigens indicates increased risk up to 5.4-fold of having current infection as well as increased risk up to tenfold of having atrophic gastritis (Shafaie et al. 2018). Seropositivity for *H. pylori* VacA has been reported in colorectal cancer in African Americans (Butt et al. 2018).

Combined test of *H. Pylori* antibodies and Gastro Panel® biomarker (pepsinogen I + II and gastrin- 17) were evident as superior over serology and UBT. Investigators concluded that Gastro Panel® has best diagnostic value in *H. Pylori* infection in atrophic gastritis as well as acid- free stomach in patient with higher susceptibility towards gastric cancer (Syrjänen et al. 2019).

6.4.7 Stool Antigen Tests (SATs)

Monoclonal antibodies are frequently used in stool antigen tests (SATs) as early diagnostic tests before treatment as well as after treatment. Advancement in SAT by Thermo Fisher Sc., Waltham, USA, Amplified IDEIA Hp StAR®, exhibited excellent performance results in terms of accuracy both pre-treatment and post-treatment; hence, authors thought that IDEIA Hp StAR® could be a good choice in place of UBT in testing *H. pylori* infection and mediated gastric complications (Moubri et al. 2018).

Another advancement in SAT by Mizuho Medy, Tosu, Japan, Quick Chaser *H. pylori*® (QCP), is based on ICT (immunochromatography). In this test, the flagellar protein of *H. pylori* is targeted for ICT. QCP shows a similar performance but with a significantly higher sensitivity when compared to Testmate Rapid Pylori Antigen ® (ICT based on catalase enzyme) developed by Wakamoto Ph., Tokyo, Japan. Besides these, QCP mitigates cross-reactivity and false results with other various microorganisms than *H. pylori* in microbial gut flora (Kakiuchi et al. 2019). Testmate Rapid Pylori Antigen is generally performed in gastric juice for detection of *H. pylori* during esophagogastroduodenoscopy; however, the little number of cases and references limited its use (Kawano et al. 2018).

6.5 *Helicobacter pylori* Infection: Therapy

With the declining eradication therapy efficacy compared to traditional methods due to drug resistance, new treatments and novel paradigms should come in existence to eradicate the *H. pylori* infection. In 2019, several areas were addressed to overcome the problem in eradication therapy via various approaches. The following therapies are frequently used in *H. pylori* infection.

6.5.1 Dual Therapy

It is generally a long course of PPI and amoxicillin and a less complex therapy. This 14-day treatment shows to effectively eradicate with 92% efficacy; however, reducing the number of days to half simultaneously reduces the eradication efficacy to 87% (Tai et al. 2019).

6.5.2 Triple Therapy

A traditional triple therapy includes PPI, amoxicillin, and clarithromycin. A complicated triple therapy with multiple drug regimens on daily dosing has been reported to improve the eradication treatment of *H. pylori*. In India, a single-dose triple therapy improves the eradication state up to 90% over traditional dosing (Shahbazi and Vahdat 2018). Clarithromycin resistance has been reported as a major problem in various countries. In Japan, metronidazole is prioritized over clarithromycin as first-line regimen for 7 days with 98% eradication efficiency rather than the 60% by metronidazole in triple therapy (Mabe et al. 2018). In China, significant cure rates of 89% and 82% were reported for 14-day and 7-day triple therapy with the addition of bismuth (Leow et al. 2018).

6.5.3 Quadruple, Concomitant, Sequential, and Hybrid (QCSH) Therapies

Various original experiments evaluating concomitant therapy prove it superior over triple therapy as the first-line treatment for 5–10 days; however, a nonsignificant result was observed for 14-day therapy (Chen et al. 2018b). In Spain, a cooperative study shows concomitant quadruple therapy with 98% eradication rate; however, bismuth-based quadruple therapy shows 94% eradication rate (Macías-García et al. 2019).

A hybrid therapy has been developed through combining concomitant and sequential regimes. Due to complexity of hybrid therapy, a simple reverse hybrid therapy (14-day treatment with PPI and amoxicillin and then 7-day treatment with metronidazole and clarithromycin) came in existence with 96% eradication rate. A similar result has also been reported in bismuth-based quadruple therapy (Hsu et al. 2018). The eradication rate in drug-resistant cases (95% for levofloxacin, 79% for clarithromycin, and 67% for metronidazole) was only 52% for 14-day quadruple therapy (Huang et al. 2018).

A sequential therapy has been designed to mitigate the problem of antibiotic resistance by *H. pylori* in eradication therapy. Sequential therapy for 10 days shows success rate of 93% for metronidazole and clarithromycin-, 96% for metronidazole-, 93% for clarithromycin-, and 83% for both metronidazole and clarithromycin-resistant *H. pylori* (Gatta et al. 2018).

6.5.4 Levofloxacin-Based Therapy

Levofloxacin for *H. pylori* treatment in the bismuth quadruple regimen was found to be 79% and 77% effective with 200 and 500 mg doses, respectively, in China (Gan et al. 2018). Higher doses provide inferior results with side effects. Levofloxacin in sequential regimen therapy for 10 days has a 78% cure rate as compared to 83% in

quadruple therapy for 14 days (Hajiani et al. 2018). Levofloxacin-based triple therapy for 14 days against *H. pylori* shows a similar result, 92% potential as compared to 87% in clarithromycin-mediated triple therapy (Latif et al. 2018).

Addition of bovine lactoferrin as an adjuvant to levofloxacin in triple therapy shows a 96% cure rate; however, without lactoferrin, the cure rate was 75% against *H. pylori* infection (Ciccaglione et al. 2019). Lactoferrin has been reported to have antimicrobial properties dependent on dose (Sue et al. 2019).

6.5.5 Bismuth-Based Therapy

Eradication therapy for *H. pylori* has been considered to be an effective method to mitigate gastric and colorectal cancer. Several regimens were trialed, and their effectiveness was concluded; however, addition of bismuth in therapy was found to significantly improve the eradication efficacy in different regimens. HpEuReg (European Registry on the Management of *H. pylori* Infection) reported that first-line triple therapy including the bismuth cured 88% of the patients (McNicholl et al. 2020; McNicholl et al. 2019). Generally, effectiveness of various drug regimens with bismuth was reported in 10 days of therapy, but 7-day treatment has also a 94% eradication rate when compared with moxifloxacin-mediated 14-day eradication therapy (Kim et al. 2019).

In China, several experiments have been performed using bismuth as a chief component in various drug regimens. Naïve patients undergoing the bismuth-based quadruple regimen were cured with 88% effectiveness (Guo et al. 2019). A triple-regimen therapy in a single capsule known as Pylera® consisting of bismuth and two antibiotics has also been reported to exert significant results along with PPI. Changes in antibiotics, amoxicillin and clarithromycin in place of metronidazole and tetracycline, do not show significant differences in results (Xie et al. 2018). It has been found that bismuth increases the curing percentage when used in various drug regimens in drug resistance cases also (O'Connor et al. 2019).

In Italy, Pylera® is generally recommended in sequential therapy and quadruple therapy for *H. pylori* eradication, showing 91% and 92% of eradication efficacy, respectively (Fiorini et al. 2018a, 2018b). Despite the successful results of bismuth-based therapy against *H. pylori,* its negative effects and failure results have also been observed in patients >60 years of age in Korea, indicating further research in older patients (Lee et al. 2019a).

6.5.6 Rifabutin, Furazolidone, and Sitafloxacin

With the development of resistance against common antibiotics, there is necessarily a need of antibiotics or various antibiotic regimens. Furazolidone, rifabutin, and sitafloxacin have been reported to be effective against over 100 strains of *H. pylori*

in Bangladesh and Nepal that exhibit resistance against metronidazole, levofloxacin, and clarithromycin. Sitafloxacin susceptibility was reported as 98% in Bangladesh and 95% in Nepal cases (Miftahussurur et al. 2019a). Similar results were recorded for 63 resistant strains in a study from the Dominican Republic (Miftahussurur et al. 2019b).

6.5.7 Probiotics

Probiotics use in eradication therapy of *H. pylori* has been reported to reduce the side effects of the therapy while simultaneously helping the eradication rate. An investigation carried out in Spain invloving 10- day triple therapy or non-bismuth quadruple concomitant therapy with Pediococcus acidilactici and Lactobacillus plantarum as probiotics results no differences between probiotics and non-probiotic groups (McNicholl et al. 2018).

Use of probiotics such as *Saccharomyces boulardii* sachets and *Lactobacillus* tablets with bismuth-based therapy is reported to reduce the side effects of the therapy such as diarrhea, constipation, and abdominal distension (Zhu et al. 2018). It is found that bismuth quadruple regimen therapy with probiotics (*Lactobacillus* with other various strains) is a highly significant combination for *H. pylori* eradication without any side effects of the therapy; however, three-in-one bismuth quadruple therapy with probiotics failed (Zagari et al. 2018).

6.5.8 Vonoprazan

Vonoprazan-based therapy is introduced by two simultaneous trials in Japan: (1) vonoprazan- based triple therapy showed 97% effectiveness in first-line patients (N = 1355) in spite of 86% of them receiving PPI-based triple therapy against *H. pylori* (Tanabe et al. 2018), and (2) vonoprazan-based first-line triple therapy was 91% effective as compared to 85% of standard therapy. However, for second-line therapy, they show 87% and 88% effectiveness, respectively (Mori et al. 2018). Vonoprazan is a potassium-competitive acid blocker (P- CAB) that inhibits gastric acid secretion by modulating H^+/K^+-ATPase activity reversibly. Vonoprazan has been thought to be a replacement of PPIs due to its higher inhibition activity with beneficial effects than PPIs in *H. pylori* eradication therapy (Sunwoo et al. 2018). Meta-analysis explains that vonoprazan-based therapy against *H. pylori* (clarithromycin susceptible) shows similar results as conventional PPI-based therapies (95% vs. 93%); however, vonoprazan-based therapy is highly significant (82% vs. 40%) than PPI-based therapies against *H. pylori* strains resistant to clarithromycin (Li et al. 2018a).

6.6 Antibiotic Resistance

Emerging antibiotic resistance in *H. pylori* is a major challenge in eradication therapy. A meta-analysis of over 178 articles from 65 countries shows ≥15% 1° and 2° resistance to drugs levofloxacin, metronidazole, and clarithromycin. America and the Southeast Asia regions show 10% 1° resistance to clarithromycin (Savoldi et al. 2018). Table 6.1 summarized the resistant drugs and their study details.

6.7 Impact of *H. pylori* Eradication on Gut Microbiota

Various studies provided a mixed conclusion of short-term changes as well as long-term changes. However, at the end of eradication therapy, bacterial diversity has been reported to be significantly reduced (Jakobsson et al. 2010; Oh et al. 2016). Triple therapy reduces the abundance of *Firmicutes* but increases *Proteobacteria*; however, bismuth quadruple therapy also increases *Proteobacteria* but reduces

Table 6.1 Resistance (percentage) of *H. pylori* against various drugs in therapies in several countries

Country and sample size (*N*)	% resistance against the drugs					Reference
	CLA	MET	AMO	TET	Quinolone	
China (*N* = 1117)	22.1	78.2	3.4	1.9	19.2 (LVX)	Liu et al. (2018a)
Italy (*N* = 1424)	35.9	40.2	0.06	–	39.3 (LVX)	Fiorini et al. (2018a, 2018b)
Algeria (*N* = 270)	29.7	46.7	5.2	2.6	17.9 (CIP); 17.2 (MOX)	Bachir et al. (2018)
Portugal (*N* = 2194)	42.0	25.0	0.1	0.2	18.0 (LVX); 9.0 (CIP)	Lopo et al. (2018)
USA (*N* = 800)	29.8	42.8	–	–	14.1 (LVX)	Mosites et al. (2018)
Iran (*N* = 2018)	34.4	79.4	27.1	38.5	58.7 (OFX); 46.8 (CIP); 45.0 (LVX)	Saniee et al. (2018)
Vietnam (*N* = 2318)	34.1	69.4	15.0	17.9	27.9 (LE V)	Khien et al. (2019)
Japan (*N* = 208)	48.0	49.0	13.0	–	–	Kageyama et al. (2019)
Poland (*N* = 170)	46.0	56.0	–	–	6.0 (LEV)	Bińkowska et al. (2018)
South Korea (*N* = 74)	31.0	41.8	6.7	–	39.2 (MOX)	Lee et al. (2019b)

Abbreviations: AMO amoxicillin, *CIP* ciprofloxacin, *CLA* clarithromycin, *LVX* levofloxacin, *MET* metronidazole, *MOX* moxifloxacin, *OFX* ofloxacin, *TET* tetracycline

Actinobacteria and *Bacteroidetes*. In addition, bismuth quadruple therapy and concomitant therapy altered α-diversity and β-diversity to a larger extent with respect to triple therapy (Chen et al. 2018a). Within 8 weeks, microbiota diversity was restored in patients receiving triple therapy; however, bismuth quadruple therapy and concomitant therapy were reported to take more than 1 year for full recovery of α-diversity and β-diversity (Chen et al. 2018a). Besides microbiota diversity restoration, eradication therapy exerts beneficial cellular activities in 8 week to 1 year, viz., reduction in TAG lipids and insulin resistance while simultaneously improving the good cholesterol (HDL) level (Chen et al. 2018a).

6.8 Vaccines

The highly conserved sequences of various molecules in *H. pylori* could work as immunogenic substances. Previously, Lpp20 lipoprotein has been recognized as anti-pylori infection in mice (Keenan et al. 2000). In Lpp20, two immunodominant epitopes have been recognized to stimulate and proliferate the CD4+ T-cells (Ning et al. 2018). Another peptide having a sequence MVTLINNE from *H. pylori* immunogenic protein (50–52 kDa) has been recognized as a promising sequence for vaccine due to stimulating proliferation of splenic lymphocytes (Espinosa-Ramos et al. 2019).

Various researches hypothesized that combining several antigens could be more efficient rather than the preparation of a single antigen. Several adjuvants have promising effects in *H. pylori* vaccine development eliciting mucosal immunogenic potential, especially heat-labile enterotoxin (LT) of *Escherichia coli* and cholera toxin (CT) (Stubljar et al. 2018; Sutton and Boag 2018). Besides these, LPS, CpG, and CF are also effective adjuvants facilitating antigen uptake and inducing protective immunity.

Ghasemi et al. synthesized a fusion protein (rFUVL) isolated from several antigenic components of *H. pylori*, viz., VacA, urease B subunit (UreB), flagellar hook-associated protein 2 (FliD), and CagL. This fusion protein as a vaccine could be administered orally or subcutaneously. In the oral route, rFUVL was administrated with cholera toxin subunit B (CTB); however, subcutaneously, it is co-administrated with AddaVax (squalene-based oil-in-water nano-emulsion [NE]) or CpG oligodeoxynucleotides. This fusion vaccine administered in mice significantly reduced one-third of bacterial strength in the stomach. The underlying reason for this effect was suggested as antigen-specific production of IgG2a/IgG1 as well as mixed responses of Th1/Th2/Th17 (Ghasemi et al. 2018). No adverse toxic effects have been recognized in the fusion protein rFUVL in any mode of administration making it a promising vaccine against *H. pylori*.

Another vaccine CTB- HUUC, derived from urease A & B subunit (UreA, UreB), HpaA and CagA conjugated with CTB adjuvant induces the mucosal antibody responses and specific serum against *H. Pylori* as compare to the immunogenic effects of PBS or CBT alone (Pan et al. 2018).

6.8.1 Optimization of the Delivery System

Intranasal immunization has been reported to be a good delivery system for P22 peptide (MEGVLIPAGFIKVTILEP) with slight modification. Yang et al. used adjuvants (nano- emulsion, NE) for P22 to strongly induce mucosal immune responses without exhibiting cytotoxicity through enhancing specific memory T-cell responses (Yang et al. 2018). Intranasal immunization prolongs the stay time of the antigen in the nasal mucosa and hence the epitope uptake capacity by mucosal cells to boost Th1 responses against *H. pylori* colonization. Gastric inflammation has also been found to be simultaneously reduced in NE-P22-immunized mice vs. mice immunized with P22 or NE alone. The P22 epitope immunogenicity should also be tested in humans (Yang et al. 2018). Liu et al. used HP55/poly(n-butylcyanoacrylate) (PBCA) NPs to make an oral *H. pylori* subunit vaccine (CCF) having the ability to withstand the harsh acidic environment of the gastrointestinal tract as well as against proteolysis (Liu et al. 2018b).

6.8.2 Clinical Trial Vaccine

It is not easy to translate the findings on effects of vaccine in mice to humans. CagA and VacA antigens induce protective immunity against *H. pylori* in mice, but they are well tolerated while strongly eliciting memory responses in naïve volunteers for *H. pylori* infection. It has been found that the subunit vaccine with NapA fails to protect the volunteers challenged by CagA+ *H. pylori* in phase ½ clinical trial (Malfertheiner et al. 2018). Further research is needed to understand immunity against natural *H. pylori* infection in the development of vaccines against various *H. pylori* strains.

6.9 Conclusion

The American Cancer Society funded 78 grants till August 2019 for $25 million for colorectal cancer considering severe health challenges. In conclusion, there are several evidences supporting the association of *H. pylori* and colorectal cancer mediated through other gastric disorders. *H. pylori* initiates a complex multifactorial process leading to colorectal polyps and thus colorectal cancer. Eradication of *H. pylori* infection has been reported to reduce the risk of *H. pylori*-mediated colorectal cancer and various gastric disorders. However, advancement in drug resistance by bacteria challenged eradication therapy and hence colorectal cancer treatment. Developing vaccines have different issues and challenges. Further in-depth studies with rigorous methodologies are needed to find the transparent mechanism of *H. pylori* in progressive colorectal tumorigenesis.

Conflict of Interest None

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Chapter 7
Diagnosis of Colorectal Cancer Using Molecular Techniques

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Abstract Colorectal carcinoma is a leading cause of death among both males and females globally, with the prevalence being higher in developed countries. It maybe hereditary, sporadic or familial. A complex array of molecular and genetic pathways is causal. The Fearon-Vogelstein adenoma-carcinoma multistep model of colorectal carcinoma is one of the best-known carcinogenesis models involving mutations in tumour suppressor genes, loss of function of mismatch repair genes, and gain of function of proto-oncogenes. While the treatment of colorectal carcinoma with target-specific personalized therapies has improved, it is often detected at an advanced stage, thereby decreasing overall survival and disease-free survival of patients post-operatively. Owing to the progression of colorectal carcinoma from benign adenomatous polyps, screening in asymptomatic and high-risk populations is key. Thus, molecular testing has a significant role in detection of these genetic

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alterations. Molecular markers, apart from being diagnostically important, also help personalize adjuvant therapy as molecular analysis via validated microarray gene expression profiles provides improved recurrence risk determination and median overall survival via targeted therapy to certain genotypes of colorectal carcinoma. This chapter aims at providing insights on the pathogenesis of colorectal carcinoma, traditional and newer diagnostic methodologies, molecular biomarkers and potential treatment strategies for colorectal carcinoma.

Keywords Colorectal carcinoma · molecular markers · FAP · HNPCC · *APC* gene · oncogenes · epigenetics

Abbreviations and Acronyms

5-FU	5-Fluorouracil
ACTRII	Activin receptor type II
ADP	Adenosine diphosphate
AFAP	Attenuated familial adenomatous polyposis
Anti-PD-1 agents	Anti-programmed death-1 agents
APC	Adenomatous polyposis coli
APAF-1	Apoptotic protease activating factor-1
ASDR	Age-standardized death rate
ASIR	Age-standardized incidence rate
ATP	Adenosine triphosphate
BAX	BCL-2- associated X gene/protein
BCL10	B-cell lymphoma/leukemia 10
BFB	Breakage-fusion-bridge
BLM	Bloom syndrome protein
BMPR1A	Bone morphogenetic protein receptor type 1A
BRAF	B-raf proto-oncogene
BUB1	Budding uninhibited by benzimidazoles 1 homolog
BUB3	Budding uninhibited by benzimidazoles 3 homolog
BUBR1	BUB1-related kinase
CA 19-9	Cancer antigen 19-9
CA 125	Cancer antigen 125
CDC4	Cell division control protein 4
CDX2	Caudal type homeobox 2
CG dinucleotides	Cytosine-guanine dinucleotide
CIMP	CpG island methylator pathway
CIN	Chromosomal instability
cMYC	Cellular myelocytomatosis oncogene
CRC	Colorectal cancer
cSRC	C-terminal SRC
DCC	Deleted in colorectal cancer

DNA	Deoxyribonucleic acid
DPC4	Deleted in pancreatic cancer-4
EGFR	Epidermal growth factor receptor
EPCAM	Epithelial cellular adhesion molecule
erbB2	v-erb-b2 avian erythroblastic leukemia viral oncogene homolog 2
FAP	Familial adenomatous polyposis
FDA	Food and Drug Administration
FGFR	Fibroblast growth factor receptor
FOLFIRI	Folinic acid, fluorouracil and irinotecan regimen
gFOBT	Guaiac-based fecal occult blood testing
GSK-3β	Glycogen synthase kinase- 3 beta
GTPase	Guanosine triphosphatases
HDI	Human development index
hMLH1	Human MutL homolog 1
hMSH2	Human MutS homolog 2
hMSH3	Human MutS homolog 3
hMSH6	Human MutS homolog 6
HNPCC	Hereditary Non-Polyposis Colon Cancer
hnRNPK	Heterogenous nuclear ribonucleoprotein K
hPMS1	Human PMS1 homolog 1
hPMS2	Human PMS1 homolog 2
HSP90	Heat shock protein 90
IGFIIR	Insulin-like growth factor 2 receptor
IgG2	Immunoglobulin G2
KRAS	Kirsten rat sarcoma 2 viral oncogene homolog
KIT	KIT proto-oncogene, receptor tyrosine kinase
LOH	Loss of heterozygosity
MAD2	Mitotic arrest deficient 2
MAPK	Mitogen activated protein kinase
miRNA	MicroRNA
MLH1	MutL homolog 1
MLPA	Multiplex ligation-dependent probe amplification
MMR	Mismatch repair
MRE11	Meiotic recombination homolog
mRNA	Messenger RNA
MSI	Microsatellite instability
MSI-H	High levels of microsatellite instability
MSI-L	Low levels of microsatellite instability
MS-S	Microsatellite stable
MYC	Myelocytomatosis oncogene
MYH	MutY homolog
NCCN	National Comprehensive Cancer Network
nCIN	Numerical chromosomal instability
NRAS	Neuroblastoma RAS viral oncogene homolog
p38α	Mitogen activated protein kinase 14 (MAPK 14) gene

p53	Tumour protein (TP) 53
PCNA	Proliferating cell nuclear antigen
PDGFR	Platelet-derived growth factor
PI3K	Phosphoinositide 3-kinase
PKC	Protein kinase c
PMS2	PMS1 homolog 2
POLD1	Polymerase delta 1
POLE	DNA polymerase epsilon
PTEN	Phosphatase and tensin homolog
RE	Restriction endonuclease
RET	Rearranged during transfection
sCIN	Structural chromosomal instability
SDI	Sociodemographic index
SMAD2	Mothers against decapentaplegic homolog 2
SMAD4	Mothers against decapentaplegic homolog 4
SSA	Sessile serrated adenoma
STK11	Serine/threonine-protein kinase STK11
STK15	Serine/threonine-protein kinase STK15
Tcf/LEF	T Cell factor/ Lymphoid Enhancer Factor
TGFβ	Transforming growth factor beta
TGFβRII	Transforming growth factor beta receptor II
TIE2	Tyrosine-protein kinase receptor Tie-2
UDP	Uridine diphosphate
UGT1A1	UDP glucuronosyltransferase 1 family, polypeptide A1
VEGFβ	Vascular endothelial growth factor beta
VEGF-A	Vascular endothelial growth factor A
VEGFR1	Vascular endothelial growth factor receptor 1
VEGFR2	Vascular endothelial growth factor receptor 2
VEGFR3	Vascular endothelial growth factor receptor 3
WISP-3	WNT1-inducible-signaling pathway protein 3
Wnt	Wingless-related integration site
ZW10	Centromere/kinetochore protein zw10 homolog
ZWILCH	Protein zwilch homolog

7.1 Introduction

Cancer is a disease characterized by the unchecked division of abnormal cells. When this type of growth occurs in the colon or rectum, it is called colorectal cancer (CRC) (American Cancer Society 2020). Together, the colon and the rectum make up the large intestine. The ileum, which is the last part of the small intestine, connects to the cecum, the first part of the colon, in the lower right abdomen and the rest of the colon is divided into four parts:

1. The ascending colon which extends up to the right side of the abdomen.
2. The transverse colon which traverses the abdomen from right to left and along with the ascending colon, it makes up the proximal colon.
3. The descending colon descends on the left side of the abdomen.

The sigmoid colon, named for its "S" shape, is the final portion of the colon and along with the descending colon it is referred to collectively as the distal, or left, colon (Fig.7.1). After the final sigmoidal segment of the colon, the large intestine concludes with the rectum which terminates in the anus.

This anatomy is of key importance because tumours within the colorectum vary in their molecular, biological, and clinical features, and in their association with risk factors (American Cancer Society 2020).

Cancer is fundamentally a disease in which the clonal accumulation of genetic alterations by the cell allows uncontrolled growth, evasion of cell death, local invasiveness and metastatic potential (Fearon and Vogelstein 1990; Nowell 1976; Vogelstein et al. 1988) and no cancer better exemplifies our current knowledge of the molecular basis of neoplasia than colorectal cancer. Recent advances in molecular biology are revolutionizing medicine. New information is being translated rapidly into clinical use, with the development of new prognostic and predictive markers and new biologic therapies. Increasingly, personalization of cancer therapy and incorporating information about each patient's tumour characteristics, individual genome, tumour microenvironment and host immune responses is considered into clinical decision making (Andersen et al. 2019). This chapter, thus, hopes to shed light upon its molecular diagnostic, prognostic and treatment aspects.

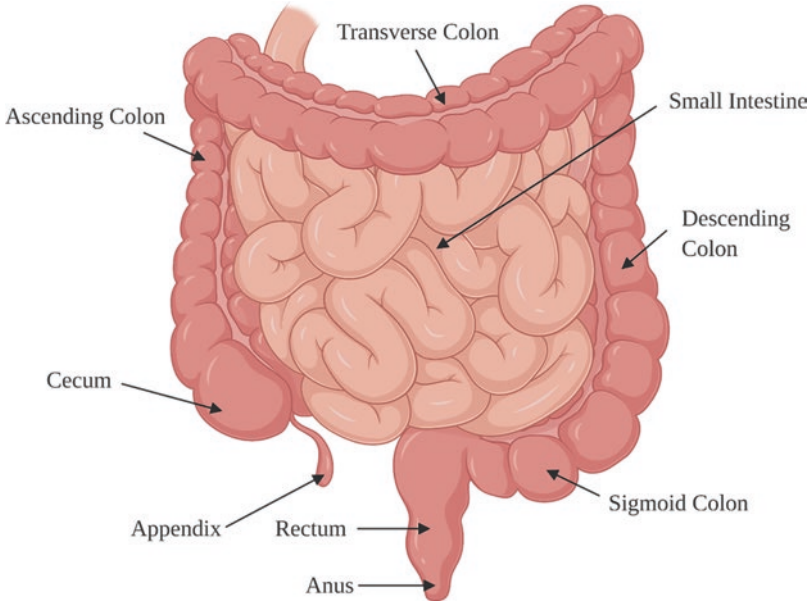

Fig. 7.1 Anatomy of the colon and rectum. (Created with BioRender.com)

7.2 Epidemiology

Cancer of the colon and rectum is a prevalent disease of the western world. Worldwide, CRC accounted for an estimated 1.85 million new cases and 881,000 deaths in 2018. The global disease burden in 2016 was estimated as 17.2 million (95% confidence interval, 16.5–17.9 million) disability-adjusted life years, of which 97% came from years of life lost due to premature mortality and 3% came from years of healthy life lost due to disability (Senore et al. 2020; Bray et al. 2018). Colorectal adenocarcinoma is the third most common cause of cancer-related deaths in males and females in the United States (Jemal et al. 2010). Colorectal cancer rates show a strong positive gradient with Human Development Index (HDI) or Sociodemographic Index (SDI) (Senore et al. 2020; Fidler et al. 2017). CRC tends to occur more frequently in men than in women, although the male to female ratio decreases from 1.6 in countries with high SDI to 1.0 in countries with low SDI (Senore et al. 2020).

The estimated age-standardized incidence rates (ASIR) of colorectal cancer in countries with a higher HDI about five times those in countries with lower capital HDI. In Australia and Europe the rates are 35–42 per one lakh in men and 24–32 per one lakh in women, compared with 7 per one lakh in men and 6 per one lakh in women in West Africa and 6 per one lakh in men and 4 per one lakh in women in South Asia (Senore et al. 2020). Despite being a prevalent disease of developed nations, there has been a rise in incidence of CRCs in developing nations in the recent years. As cancer is a disease of the ageing, the rates of CRC development and death increase rapidly after the age of 50 years with an estimated 90% of global cases and deaths in this age group (Bray et al. 2018; Duff and George 2015).

Colorectal cancer occurs in hereditary, sporadic and familial forms. It is a highly preventable disease with a substantial proportion of the burden attributed to modifiable lifestyle and environmental factors. An improved understanding of the biology, natural history of CRC, effective screening at the appropriate time and diagnosis at an earlier stage can contribute to improving the outcomes (Senore et al. 2020).

7.3 Risk Factors

The risk factors of the disease are as follows

7.3.1 Ageing

Ageing is a dominant risk factor for CRC with more than 50% cases diagnosed in the fifth decade of life. However, this does not eliminate the chance that individuals of any age group can develop CRC (Bray et al. 2018).

7.3.2 Gender

The incidence of colon cancer is similar in men and women, but rectal cancer is more prominent in men (Senore et al. 2020; Bray et al. 2018).

7.3.3 Ethnicity

CRC is more common in African Americans than in Caucasians with a 45% higher mortality (Augustus and Ellis 2018).

7.3.4 Hereditary Factors

80% of CRCs occur sporadically, that is in the absence of a positive family history. However, 20% of cases arise in patients with a positive family history of CRCs (Mishra and Hall 2012). Thus, increasing the importance of early diagnosis using genetic testing (Table 7.1).

Personal history of adenomatous polyps is associated with an increased risk of CRC based on the type of polyp with tubular adenomas at the lowest risk, tubulovillous adenomas having an intermediate risk and villous adenomas having the highest risk of transformation to CRC (Duff and George 2015).

7.3.5 Environmental and Dietary Factors

Diets rich in saturated or polyunsaturated fats increase the risk of CRCs, while a diet rich in oleic acids (olive oil, coconut oil, fish oil) is considered protective. A high fibre diet is also considered to be protective. Calcium, oral bisphosphonate therapy

Table 7.1 Familial risk and colon cancer (Burt 2000)

Familial setting	Approximate lifetime risk of colon cancer
General U.S. population	6%
One first-degree relative (parents, siblings or children) with colon cancer	2- to 3-fold increased
Two first-degree relatives with colon cancer	3- to 4-fold increased
First-degree relative with colon cancer diagnosed at ≤50 years	3- to 4-fold increased
One second-degree relative (grandparents, aunts or uncles) or third-degree relative (great-grandparents and cousins) with colon cancer	1.5-fold increased
Two second- or third-degree relatives with colon cancer	2- to 3-fold increased
One first-degree relative with adenomatous polyp	2-fold increased

for at least 1 year's duration, selenium, vitamin A, C and E, carotenoid supplementation and plant phenols are considered protective against CRC. Studies suggest a positive correlation between alcohol intake, cigarette smoking, and CRC. Obesity and a sedentary lifestyle dramatically increase cancer-related mortality (Andersen et al. 2019; Calle et al. 2003).

7.3.6 Inflammatory Bowel Disease and Primary Sclerosing Colingitis

The degree, duration, and extent of inflammation is directly proportional to the risk of development of CRC. Ulcerative colitis increases the risk by seven- to eleven-fold, whereas, Crohn's disease is associated with a two-fold increased risk of CRC (Duff and George 2015).

7.3.7 Other Risk Factors

Patients with ureterosigmoidostomy, acromegaly and a history of pelvic irradiation at a risk for both adenoma-carcinoma formation (Andersen et al. 2019). According to the American Cancer Society, there is a lower risk of CRC in regular, non-steroidal anti-inflammatory drug users which indicates some protective factor (American Cancer Society 2020).

7.4 Current Screening Guidelines of CRC for Asymptomic, Average-Risk Individuals

Beginning at the age of 50 years, all patients should have *one* of the required screening options (Table 7.2) (Duff and George 2015).

Table. 7.2 Screening tests for CRC (Duff and George 2015)

Test	Frequency
Guaiac-based fecal occult blood testing (gFOBT) (every 3 years) + flexible sigmoidoscopy (every 5 years)	Ongoing.
gFOBT or fecal immunochemical test (FIT)	Every year.
Colonoscopy- In case of a positive FOBT, polyp on sigmoidoscopy, abnormal double contrast barium enema study	Every 10 years. Polyps to be removed completely during colonoscopy.
Flexible sigmoidoscopy	Every 5 years.
Double contrast barium enema	Every 5 years.

7.5 Molecular Basis of CRC

The field of CRC was revolutionized in 1988 by the description of the genetic changes involved in the progression of a benign adenomatous polyp to invasive carcinoma. The Fearon-Vogelstein adenoma carcinoma multistep model of colorectal neoplasia resembles one of the best-known models of colorectal neoplasia (Fearon and Vogelstein 1990; Vogelstein et al. 1988; Ivanovich et al. 1999).

Malignant transformation is the process by which a clonal population of cells undergoes changes that enable them to grow over normal cells (Fig.7.2). Most of these mutations occur at the genetic level and are a product of either the activation of oncogenes or deactivation of tumour suppressor genes (Townsend et al. 2016).

Genetic mutations inherited from one's parents are called germline or constitutional mutations which are present in all the cells of the body, whereas, mutations acquired during an individual's lifetime, which cannot be passed on to one's offspring are termed as somatic mutations. Somatic mutations account for most of the mutations in CRC, as is the case for most cancers. Hereditary CRCs are a product of germline mutations with a positive family history in earlier generations. However, in some hereditary CRCs, the germline mutations may cause the cells to accumulate somatic mutations. Sporadic CRCs are noted when a patient has no inherited predisposition and tumour's genetic mutations are somatic in nature (Townsend et al. 2016).

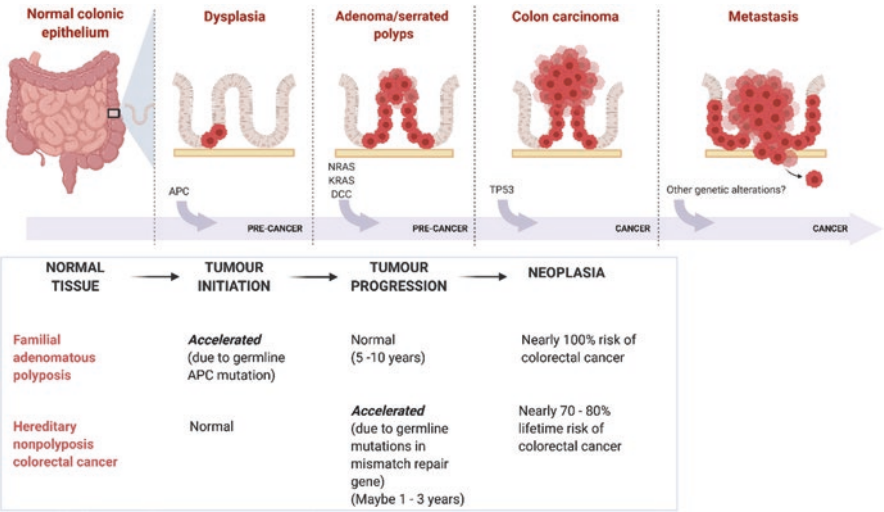

Fig. 7.2 The adenoma-carcinoma sequence in sporadic and hereditary CRC. (Modified from (Ivanovich et al. 1999). Created with BioRender.com)

7.5.1 Tumour Suppressor Genes

Tumour suppressor genes (*APC, DCC, p53*) produce proteins which inhibit the formation of a tumour by regulating mitotic activity and having an inhibitory effect over the cell cycle. This function is lost in case of genetic and epigenetic changes such as point mutations, loss of heterozygosity, frameshift mutations and promoter hypermethylation (Townsend et al. 2016).

The *APC* gene is a tumour suppressor gene located on chromosome 5q21. It was first localized in 1987 and cloned in 1991, after mutation analyses of familial adenomatous polyposis (FAP) kindreds. (Townsend et al. 2016). It forms a product which is 2843 amino acids in length. This product forms a complex with GSK-3β (a serine-threonine kinase), β-catenin and axin in the cytoplasm (Townsend et al. 2016).

Under normal circumstances, β-catenin a structural protein of the epithelial cell adherens junctions and actin cytoskeleton binds to Tcf/LEF in the cytoplasm and is then transported into the nucleus, where it is responsible for the activation of transcription of genes that regulate cellular growth and proliferation. *APC* regulates this intracytoplasmic pool of β-catenin normally. The *Wnt* signalling proteins also play a role in this *APC/*β-catenin pathway. *Wnt* gene products are extracellular signalling molecules that regulate tissue development. Normally, low intracytoplasmic β-catenin levels inhibit *Wnt* expression (Townsend et al. 2016). Mechanisms that cause mutational inactivation of *APC* earliest in the adenoma carcinoma sequence induce loss of E-cadherin functioning which is a prototype cadherin responsible for cell polarity and organization of epithelium. This prevents β-catenin from binding and leads to its intracytoplasmic accumulation. Loss of *APC* functioning and *Wnt* activation prevents β-catenin degradation and its translocation to the nucleus where genes responsible for cell proliferation and tumour progression such as *cyclin D1* and *MYC* get activated (Townsend et al. 2016). The earliest phenotypic change in a CRC is called an aberrant crypt formation and the most consistent genetic aberrations in these cells are abnormally short premature stop codon mutations known as *APC* truncations. Of the different mutations, the most common is a frameshift mutation (68%), followed by nonsense mutations (30%) and large deletions (2%) with a majority of these mutations being located at the 5′ end of exon 15 which is called the *mutation cluster region.* These biallelic *APC* germline mutations are responsible for FAP (Familial Adenomatous Polyposis), an autosomal dominant inherited disease. However, 30% of the FAP cases may present in the absence of a family history of the disease via de novo germline mutations (Townsend et al. 2016). FAP is a rare disorder affecting 1 in 10,000 live births in the United States. Its striking feature is more than 100 adenomatous polyps in the colon and rectum. 50% of the patients present with gastric and duodenal polyps with gastric polyps mostly as a result of fundic gland hyperplasia with minimal malignant potential but duodenal polyps being adenomatous and premalignant in nature. Mutations between 976 and 1067 codons are associated with an increased risk of duodenal adenomas (Bertario et al. 2003). Congenital hypertrophy of the retinal pigment epithelium is a striking feature affecting patients and is associated with mutations between codons 463 and 1387

(Nusliha et al. 2014). The risk of developing invasive cancer is virtually 100%. More than 80% of sporadic CRCs also have somatic mutations such as these *APC* truncations. The phenotype usually presents in the second or third decade of life. Untreated individuals develop CRC by the age of 35–40 years, nearly 30 years earlier than the median age for sporadic CRC. Genetic polymorphisms in the *APC* gene have been noted inCRCs affecting Ashkenazi Jews with I307 point mutations caused by the substitution of a lysine for isoleucine at codon 1307 (Locker et al. 2006). The location of the mutation within the *APC* gene determines the phenotype, thus giving rise to different FAP variants with extra-intestinal manifestations:

1. *Attenuated FAP (AFAP)*- This variant is a result of mutations close to the 3′ or 5′ ends of the gene. The affected individuals present with less than 100 adenomas with polyps in the proximal colon and the onset of CRC occurring 15 years later than patients with FAP (Townsend et al. 2016).
2. *Gardner's Syndrome*- This syndrome is caused due to mutations between codons 1403 and 1578 (Fearon 1997). It is associated with CRC, desmoid tumours, osteoma of the mandible and skull, lipomas, thyroid tumours and fibromas of the mesentery and abdominal wall, and periampullary neoplasms (Half et al. 2009).
3. *Turcot's Syndrome*- It is associated with CRCs and brain tumours such as medulloblastomas, glioblastomas and cerebellar neoplasms. Here, *APC* mutations are commonly seen in families with cerebellar medulloblastomas, whereas *MLH1* and *PMS2* mutations are predominant in families with glioblastomas (Half et al. 2009).
4. *Peutz-Jegher's Syndrome*- It is associated with non-neoplastic hamartomatous polyps throughout the gastrointestinal tract and perioral melanin pigmentation and is also associated with mutations in the *STK11* gene located in chromosome 19p (Riegert-Johnson and Boardman 2009).
5. *Familial Juvenile Polyposis*- It is associated with hamartomatous polyps throughout the gastrointestinal tract and is also associated with mutations in *SMAD4* located in chromosome 10q and mutations in *BMPR1A* (Cichy et al. 2014).
6. *Cronkite-Canada Syndrome*- It is characterized by hamartomatous gastrointestinal polyposis, alopecia, cutaneous pigmentation and atrophy of the fingernails and toenails (Kao et al. 2009).
7. *Cowden's Disease*- It is characterized by hamartomatous polyps in all three embryonal cell layers, mucocutaneous lesions, thyroid adenomas, fibrocystic disease of the breast, uterine leiomyomas and macrocephaly. It imposes a 10% risk of thyroid cancer and a 50% risk of adenocarcinoma of the breast in affected women (Ellisen and Haber 1998).

Of these hereditary cancer syndromes, FAP, Gardner's syndrome and Turcot syndrome get classified as Hereditary Adenomatous Polyposis syndromes and Cowden disease, Familial Juvenile Polyposis and Peutz-Jeghers syndrome get classified under Hereditary Hamartomatous Polyposis syndromes.

These Hereditary Hamartomatous Polyposis syndromes and the Ruvalcaba-Myhre-Smith/Bannayan-Zonana syndrome are associated with deletions in the *PTEN* (tumour suppressor phosphatase and tensin homolog) gene located on chro-

mosome 10q. Thus, genetic testing and screening for FAP among families with these mutations is of key importance in early detection of the adenomatous polyps and management prior to conversion of these polyps to CRC (Townsend et al. 2016).

First degree relatives of patients diagnosed with FAP and CRC have to undergo regular screening with flexible sigmoidoscopy starting from the age of 10 to 15 years. Post-genetic counselling and *APC* testing of the other family members, members with the *APC* mutation must undergo annual, flexible sigmoidoscopy from the age of 10 to 15 years till a polyp is identified. For those who refuse genetic testing or in those whom *APC* mutations cannot be identified, screening must begin at least from 50 years of age. The mainstay of treatment for FAP, owing to its 100% conversion to CRC, remains surgery. How radical this surgery needs to be, depends upon the involvement of the rectum with polyps. The risk for rectal carcinoma is almost three times higher in FAP patients with mutations after codon 1250 than with mutations before this codon thus, highlighting the importance of genetic testing in influencing the decision to offer an abdominal colectomy with an ileorectal anastomosis to patients with mutations seen proximal to codon 1250 with none to few polyps in the rectum on proctoscopic examinations. This is a less radical and simpler surgery as compared to a restorative total proctocolectomy with an ileal pouch anal anastomosis and eliminates potential complication such as impotence due to autonomic nerve injury and anastomotic leak (Townsend et al. 2016). All it takes is a venipuncture to identify the mutation in the DNA of family members of the affected to aid in secondary prevention of this lethal disease.

MYH-associated polyposis (MAP) syndrome is caused by mutations in the human *MutY homolog (MYH)* gene (Al-Tassan et al. 2002). The subset of patients with an attenuated phenotype resembling FAP or AFAP with a negative test for *APC* mutations come under this category. Unlike FAP, MAP has an autosomal recessive inheritance. It is phenotypically similar to AFAP. All patients with biallelic *MYH* mutations, homozygotes or compound heterozygotes for germline *MYH* mutations have a 93-fold risk of CRC. It is characterized by 100–1000 polyps throughout the colon and extracolonic manifestations such as duodenal adenomas and breast cancer. The *MYH* gene located on chromosome 1p encodes a DNA glycosylase which plays a role in the base excision repair pathway, thus preventing mutations due to oxidative damage. 50% penetrance has been noted. The mutation causes chromosomal instability and misaggregation leading to aneuploidy. Patients with MAP need to be distinguished from those with FAP or AFAP owing to its increased risk in siblings rather than in offspring. (Townsend et al. 2016).

p53, a tumour suppressor gene located on chromosome 17p, is known as the guardian of the genome as it plays a role in regulating apoptosis. It is also the most frequently mutated tumour suppressor gene in neoplasia. Normally, *p53* induces apoptosis by activating *BAX* gene after cellular damage or causes G1 cell arrest. *p53*mutations have been found to occur late in the adenoma-carcinoma sequence in 75% CRCs. The importance of noting *p53* mutations in CRCs lies in the fact that the minority of patients without these mutations have a better prognostic outcome and survival chance (Townsend et al. 2016).

SMAD2, SMAD4 and *DCC* are genes located on chromosome 18q. The *SMAD* genes are involved in the transforming growth factor β signal transduction pathway an the *DCC* gene is involved in cell-cell interactions. *DPC4* gene located adjacent to *DCC* is thought to be a tumour suppressor gene deleted in chromosome 18q mutations and *SMAD2* and *SMAD4* have been found mutated in 10% of sporadic CRCs (Townsend et al. 2016).

7.5.2 Oncogenes

Oncogenes are abnormal cellular genes contributing to cancer. The normal counterpart of such a gene is called a proto-oncogene. Oncogenes maybe growth factors (transforming growth factor β, epidermal growth factor, insulin-like growth factor), growth factor receptors (*erbB2*), intracellular signal transduction molecules (*ras, src, abl*) or nuclear transcription factors *(myc)* (Andersen et al. 2019; Townsend et al. 2016). Mutations in proto-oncogenes produce a gain of function and it is sufficient for only one of the two alleles to undergo this mutation, thus yielding an oncogene.

The *ras* proto-oncogene is located on chromosome 12 (Bos et al. 1987). Mutations in the *KRAS* gene in particular are believed to occur early in the adenoma-carcinoma sequence in 30–50% CRCs and have been found to be present in aberrant cryptic foci and adenomatous polyps (Townsend et al. 2016). Multiple pathways such as the MAPK (mitogene-activated protein kinase), PI3K (phosphatidyl inositol 3-kinase) and PKC (protein kinase C) cascades play a role in *ras* oncogenesis (Downward 2003; Karnoub and Weinberg 2008). *p38α,* a MAPK protein plays a role in tumour suppression (Chen et al. 2000; Dolado et al. 2007), whereas *p38γ* causes *ras* oncogenesis by phosphorylation and has been noted in *KRAS* mutated CRCs. *p38γ* overexpression in CRC correlates with a shortened survival and increased metastasis. *p38γ* forms a complex with heat shock protein 90 (HSP90), a highly conserved molecular chaperone which activates more than 200 client proteins and maintains the proliferative signalling. *p38γ* forms a complex with both HSP90 and *KRAS* in *KRAS*-mutated CRCs, but not in wildtype CRCs Thus, detection of *KRAS* mutations in stools is a potentially powerful screening strategy as pharmacological inhibition of the *p38γ*-activated ternary complex is a novel therapeutic target in case of *KRAS*-dependent CRC (Qi et al. 2014).

7.5.3 Mismatch Repair Genes

Mismatch repair (MMR) genes are caretaker genes which maintain the integrity of the genome by correcting errors such as nucleotide mismatches, small insertion or deletion loops produced by DNA polymerases during the process of DNA replication (Fanale et al. 2017). When mismatch repair genes undergo loss of function mutations, DNA mutations in other genes responsible for cell proliferation accumulate at an

alarming rate. These are germline defects in MMR genes (*hMLH1*- human mutL homolog 1*, hMSH2*- human mutS homolog 2*, hMSH3, hPMS1*- human post-meiotic segregation 1*, hPMS2*- human post-meiotic segregation 2 *and hMSH6).* Of these, *hMLH1* and *hMSH2* are the genes most commonly affected (Townsend et al. 2016). Mutations in these MMR genes cause Hereditary Non-Polyposis Colon Cancer (HNPCC) syndrome also known as Lynch syndrome. This is the most commonly occurring hereditary CRC syndrome in the West, accounting for 3% of all cases of CRC.

HNPCC was first recognized in 1885 by Alder S. Warden, chairman of pathology at the University of Michigan, when his seamstress pointed her strong family history of endometrial, gastric and colon cancer. A study of her family's medical records showed an autosomal dominant transmission of the cancer risk. This family (Family G) was further studied by Henry Lynch who highlighted the features of HNPCC. The mutation in *hMSH2* was responsible for malignancies in Family G. HNPCC has a relatively younger age of onset (mean 44 years), right colonic predilection, mucinous or poorly differentiated signet cell adenocarcinomas, an increase chance of synchronous and metachronous tumours and a surprisingly positive outcome post-surgery. HNPCC has been proposed to consist of two syndromes

1. *Lynch I syndrome,* which comprises early onset hereditary predisposition for synchronous and metachronous CRCs.
2. *Lynch II syndrome*, which comprises colorectal and extracolonic cancers of the endometrium, ovaries, stomach, small intestine, pancreas and transitional cell carcinomas of the ureters and renal pelvis (Andersen et al. 2019; Lynch et al. 1993).

The diagnostic criteria for HNPCC, owing to it's strong family history was referred to as the Amsterdam 3-2-1-0 criteria, later modified to the Amsterdam criteria II (Andersen et al. 2019; Vasen et al. 1999)

- Three or more relatives with an HNPCC associated cancer (colorectal, endometrial, small bowel, renal pelvis), one of whom is a first degree relative of the other two.
- At least two successive generations affected.
- At least one diagnosed case before the age of 50 years.
- Familial adenomatous polyposis excluded.
- Tumours verified by pathologic examination.

The revised Bethesda criteria has replaced the Amsterdam criteria for clinical diagnosis for HNPCC (Duff and George 2015)

- Colorectal cancer diagnosed in a patient of the age of ≥50 years.
- Presence of synchronous or metachronous HNPCC-related tumours.
- CRC tumour with microsatellite instability-high (MSI) histology in a patient of the age of ≥60 years.
- CRC in a patient with ≥1 first-degree relative with an HNPCC-related tumour diagnosed before the age of 50 years.
- CRC in a patient ≥2 first- or second- degree relatives with an HNPCC-related tumour.

hMSH6 mutations have been particularly noted in endometrial carcinomas. *PMS2* and *hMSH6* mutations lead to an attenuated form of Lynch syndrome.

The hallmark of HNPCC is microsatellite instability. Microsatellite instability exists in 10–15% of CRCs and 95% cases of HNPCC. Thus, the mainstay of diagnosis of Lynch syndrome is a detailed family history, although 20% cases are a result of spontaneous germline mutations. CRC or any HNPCC related cancers arising in an individual younger than 50 years of age should raise a suspicion for this syndrome and genetic counselling and testing is a must. However, one must note that failure to identify a causative MMR gene mutation in a patient with a history strikingly similar to Lynch syndrome does not exclude the diagnosis of the same, as is noted in 50% of the cases.

A positive test on genetic testing warrants close surveillance with colonoscopy beginning at the age of 20 years or 10 years younger than the youngest age of diagnosis, whichever comes earlier and repeated two-yearly until the age of 35 and annually thereafter. In females, periodic vacuum curettage is done at the age of 25 years with a pelvic ultrasound, testing for serum CA 125 levels and annual test for urine occult blood. There have been well-documented cases of invasive CRCs occurring 1 year after a negative colonoscopy. Comparison of the development of CRC in FAP and HNPCC patients based on the adenoma-carcinoma sequence model has clearly demonstrated an accelerated evolution from a benign polyp to invasive cancer to be a feature of the pathogenesis in HNPCC unlike FAP, thus warranting more frequent colonoscopic examinations in patients with HNPCC so that the cancer is usually picked up at a favourable stage. In female patients who have completed their families, a prophylactic total abdominal hysterectomy and bilateral salpingo-oophrectomy is recommended. At times, prophylactic colectomy for patients with Lynch syndrome has also been considered (Townsend et al. 2016).

7.6 Other Biomarkers

- The carcinoembryonic antigen CEA is a glycoprotein vital in adhesion and apoptosis of tumour cells. Its raised levels help determine tumour recurrence post-surgery. However, its levels maybe raised in other malignancies too, thus indicating low specificity (Hall et al. 2019).
- Carbohydrate Antigen (CA19–9) is an onco-fetal antigen commonly expressed in carcinomas of the gastrointestinal tract. However, it is less sensitive in detecting CRCs than CEA (Hayat 2009).
- UDP-glucuronosyl transferase (UGT1A1) genetic polymorphisms are a predictor of severe toxicity in patients receiving Irinotecan chemotherapy (Ando et al. 2002).
- Heterogeneous Nuclear Ribonucleoprotein K (hnRNPK) is normally responsible for mRNA stabilization, regulation, splicing, nuclear-cytoplasmic shuttling, translational activation, translational silencing, translational control and forming a structural component of DNA protein complexes. Overexpression of hnRNPK

increases transcriptional activity of *cMYC* reporter gene and modulates oncogene *cSRC* expression. This biomarker holds diagnostic and prognostic potential as patients with a Dukes' C tumour and hnRNPK score had a poorer prognosis compared to those with a low score (Hayat 2009).

7.7 Mutator-Phenotype Pathways of CRC

In a general sense, genetic alterations in cancer have been observed to occur "macroscopically" as alterations in chromosome number and structure and "microscopically" as nucleotide changes involving individual genes (Gryfe 2009). Similarly, both macro- and micro-epigenetic alterations have been observed in human cancers. Basal mutation rates appear to be insufficient to account for the 6000–11,000 somatic alterations experimentally estimated to be present in a colon-cancer cell genome and has prompted the hypothesis that widespread genomic (or epigenomic) instability is an essential early step in carcinogenesis (Gryfe 2009). On an average, normal human cells have been estimated to show a mutation rate of ~2.5×10^{-8} mutations/nucleotide/generation (Nguyen et al. 2018). These rates of mutation are much higher in cancer cell genomes as a result of accumulation of multiple mutations over several generations. This proposed inherent defect that makes cancer cells susceptible to genomic instability is often referred to as the mutator phenotype. Accordingly, elevation of mutation rates to levels seen in most cancerous growths in humans may occur due to cellular stress responses and/or defects in the MMR genes, genes responsible for maintaining DNA homeostasis by regulation of cell cycle checkpoints. The 'mutator phenotype' may have various manifestations, including point mutations, chromosomal instability (CIN), microsatellite instability (MSI), CpG island methylation and loss of heterozygosity (LOH) (Nguyen et al. 2018).

To summarize, the mutations in CRC pathogenesis have now been recognized to occur via the following major genetic pathways (Gryfe 2009)

1. The chromosomal-instability (CIN) pathway
2. The microsatellite instability (MSI)
3. The CpG island methylator pathway (CIMP).

7.7.1 The Chromosomal Instability Pathway

The chromosomal instability pathway is also known as the loss of heterozygosity pathway. Chromosomal instability is defined as a dynamic state in which cells continuously show an increased rate of loss or gain of large portions of chromosomes, or whole chromosomes in cancer which results in activation of growth- promoting pathways and/or decreased activity of apoptotic pathways (Gryfe 2009). Karyotypic

variability is observed in cells eventually resulting in aneuploidy, sub-karyotypic amplification, chromosomal rearrangement, and high frequency LOH at tumour suppressor gene loci (Nguyen et al. 2018). The majority of colorectal cancers are aneuploid, consistent with a chromosomal-instability pathway and driven by mutational events in oncogenes, including KRAS proto-oncogene GTPase (*KRAS*), proto-oncogene serine/threonine kinase (*BRAF*), and the tumour suppressor genes *APC* and tumour protein 53 gene (*p53*) (Nguyen et al. 2018; Centelles 2012). Mutations in these genes contribute to CRC tumorigenesis. This pathway was first described in patients with FAP with *APC* gene mutations.

Chromosomal instability tumours contain both numerical (nCIN) and structural (sCIN) chromosome changes. Numerical CIN (nCIN) is related to gain or loss of whole chromosomes and thus results in a change in chromosome number or aneuploidy. However, aneuploidy, an abnormal chromosomal number, is not synonymous to CIN, although CIN is the main cause of aneuploidy.

In contrast to a dynamic, rate defined, chromosomal-instability mechanism, aneuploidy could arise from clonal selection and expansion of cells with a normal baseline rate of chromosomal changes, but an increased rate of replication or, alternatively, as a result of exposure of cells to either an endogenous or exogenous force that creates a stable, but abnormal chromosomal content at a single point in time. Furthermore, aneuploidy could result from a basal rate of chromosomal alteration that, in a normal cell, leads to cell death but is tolerated and clonally expanded in a cancer cell (Li et al. 2000). Molecular mechanisms for nCIN include weakening of the mitotic checkpoint, aberrant sister chromatid cohesion, centrosome amplification and improper attachment of chromosomes to the mitotic spindle (Centelles 2012). Structural CIN (sCIN), on the other hand, involves an increased rate of formation of structurally abnormal chromosomes. A key feature associated with sCIN is the formation of "reactive" chromosomes after the breaking of chromosomes. These "reactive" chromosomes result in breakage-fusion-bridge (BFB) cycles, which can increase the genomic rearrangements. BFB cycles have been found to be associated with sCIN and intra-tumour heterogeneity. The molecular mechanisms of sCIN include telomere dysfunction, and aberrant DNA repair pathways (Centelles 2012). The Fearon and Vogelstein multistep genetic model for colorectal carcinogenesis is most commonly adopted, and used as a paradigm for solid tumour progression. CRCs are characterized with high frequency allelic losses at numerous chromosomal arms bearing candidate tumour-suppressor genes such as *APC* on chromosome 5q, *p53* on chromosome 17p, DCC netrin 1 receptor (*DCC*) and SMAD family members (*SMAD2* and *SMAD4*) on chromosome 18q. 1p, 8p, 18p, 20p and 22q are other common sites that can show high-frequency allelic losses. Contrary to this, the presence of oncogenes favouring cell growth and survival causes a gain of chromosomal material, most commonly seen in CRC at chromosome 7, and chromosomal arms 1q, 8q, 12q, 13q and 20q (Nguyen et al. 2018). Thus, CIN induces carcinomas through the loss or mutation of tumour suppressor genes such as *APC*, *p53* and also through activation of oncogenes such as *KRAS*. The basis for most plausible causes of colorectal cancer chromosomal instability appears to involve direct disruption of regulation of the mitotic spindle. The mitotic spindle

is part of the eukaryotic cell cytoskeleton that aligns and separates replicated chromosomes (sister chromatids) into daughter cells during mitosis. Mitotic-spindle arrest, due to abnormal chromosomal alignment, is dependent on the activity of a number of kinetochore-associated proteins including BUB1, BUB3, and MAD2 (Potter and Lindor 2009).

Proposed genetic causes of colorectal cancer chromosomal instability include (Gryfe 2009)

- Heterozygous splice-site mutations of the mitotic-spindle assembly checkpoint gene, *BUB1* and its homolog, *BUBR1* (Cahill et al. 1999).
- Loss of the *MAD2* mitotic-spindle checkpoint gene (Michel et al. 2001).
- Somatic mutations of the cyclin E regulator, *CDC4* (also known as *Fbw7*) gene which normally participates in ubiquitin-mediated proteolysis of cyclin E and regulation of the G1-S cell-cycle checkpoint (Akhoondi et al. 2007; Kemp et al. 2005; Rajagopalan et al. 2004).
- Amplification of *Aurora-A* (also known as *Aurora2* and *STK15*), another mitotic-spindle checkpoint gene, which is associated with overexpression of both mRNA and protein (Bischoff 1998; Zhou et al. 1998).
- Mutations in the DNA double-strand-break gene, *MRE11* (Wang et al. 2004).
- Mutations in a putative anaphase inhibitor gene, *Ding* (Wang et al. 2004).
- Mutations in three putative spindle checkpoint genes (*ZW10*, *ZWILCH*, and *ROD*) (Duensing and Duensing 2005).
- Inactivation of the *p53* transcription factor which leads to cell-cycle checkpoint failure and evasion of apoptosis in the presence of DNA damage. It is the most frequently mutated tumour-suppressor gene in all cancers, including colorectal cancers (Vogelstein and Kinzler 2004).
- Truncating mutations of the colorectal cancer gatekeeper gene, *APC*, play a critical role in establishing chromosomal instability in the majority of colorectal adenomas and carcinomas. It is a key regulator of mitotic-spindle assembly and the mitotic-spindle checkpoint (Kinzler et al. 1991).

7.7.2 The Microsatellite Instability Pathway

DNA mismatch repair (MMR) system corrects erroneous insertion, deletion, and base-base mismatches generated during DNA replication and recombination that have escaped the proofreading process (Nojadeh et al. 2018). The MMR system is mainly composed of five genes (*MSH2*, *MLH1*, *MSH3*, *MSH6*, and *PMS2*) that encode proteins which are critical to the proper repair of DNA sequence mismatch errors missed by DNA polymerases and the preservation of genomic integrity (Thomas and Shi 2017).

When a mismatch is detected in the eukaryotic genome, DNA mismatch repair system functions through a series of steps:

1. MSH2 associates with MSH6 or MSH3 causing the formation of MutSα and MutSβ heterodimers, respectively.
2. MutSα recognizes single base mismatches and small insertion/deletion loops (IDLs), while MutSβ recognizes larger loops.
3. MutSα or MutSβ can recruit MutLα, MutLβ or MutLγ heterodimers (if MLH1 couples with PMS2, PMS1 or MLH3, respectively) by means of exchanging adenosine triphosphate (ATP) to adenosine diphosphate (ADP).
4. This complex (MutS-MutL) creates a sliding clamp around the DNA.
5. The proteins in sliding clamp interact with exonuclease-1 and proliferating cell nuclear antigen (PCNA).
6. This complex excises the daughter strand back to the site of the mismatch.
7. Finally, resynthesize and re-ligation are performed by DNA polymerase and DNA ligase, respectively and the correction is made.

By definition, microsatellites are short repeated segments of DNA that are interspersed randomly across the human genome. They are polymorphic, both in repeat size and number. Repeating units vary in size between one (mononucleotide repeat) and six nucleotides, approximately, and contain 10–50 identical repeats per microsatellite locus. Microsatellites are, by their repetitive nature, susceptible to instability due to slippage of the DNA polymerase complex during the DNA replication process. Instability, in the form of contractions (deletions) or expansions (insertions) in repeat length, occurs when the DNA MMR mechanism fails to correct these mutations (Potter and Lindor 2009). Examples of genes containing coding repeats that are targets for mutation in CRC with MSI include genes related with DNA repair (*RAD50*, *MSH2*, *MSH3*, *MSH6*, *MLH1*, *BLM*, *PMS2*), apoptosis (*APAF1*, *BAX*, *BCL10*, *Caspase-5*), signal transduction (*TGFβRII*, *ACTRII*, *IGFIIR*, *WISP-3*), cell cycle (*PTEN*, *RIZ*), and transcription factors (*TCF-4*) (Centelles 2012).

Microsatellite instability in tumour DNA is defined as the presence of alternate sized repetitive DNA sequences that are not present in the corresponding germline DNA, a molecular phenotype arising due to a defective DNA mismatch repair system (Nojadeh et al. 2018). Standard designations that describe the various levels of microsatellite instability within colon tumours have been formally adopted as follows: MSI-H (high level of microsatellite instability), MSI-L (low level of microsatellite instability), and MS-S (microsatellite stable). MSI-H tumours are characterized by instability detected at 30% or greater of the microsatellite markers analysed. MSI-L describes tumours that demonstrate instability at less than 30% of markers tested, and MS-S tumours are characterized by stability of all markers tested. Mutational inactivation or epigenetic inactivation through CpG island methylation of promoter sequences results in the loss of MMR gene function(Nguyen et al. 2018). These defects in the mismatch repair system increase the occurrence of mutations due to replication errors which subsequently increases the possibility of development of malignancies. Majority of MSI-H cancers are positive for *MLH1* promoter hypermethylation, while mutations in both *MLH1* and *MSH2* are relatively less common (Nguyen et al. 2018). Frameshift mutations due to small insertions and deletions in certain repeat sequences of coding regions of essential

oncogenes and tumour-suppressor genes can contribute to MSI-H CRC tumorigenesis (Nguyen et al. 2018). Tumours associated with MSI have diverse biologic characteristics from tumours resulting due to LOH pathways as tumours with MSI are likely to be in the proximal colon and possess diploid DNA and have a better prognosis, whereas tumours arising from the LOH pathways occur in the distal colon possess chromosomal aneuploidy and have a poorer prognosis.

7.7.3 The CPG Island Methylator (CIMP) Pathway

DNA methylation, an epigenetic modification that regulates gene expression, is required for normal embryogenesis, X-chromosome inactivation and genomic imprinting (Thomas and Shi 2017). CpG islands are regions of nucleic acid that are often located proximally to the transcription start site of genes that contain a high frequency of CG dinucleotides. CIMP involves the transcriptional repression of tumour suppressor genes. This suppression is associated with abnormal methylation of nucleic acid at certain cytosine residues of the cytosine and guanine-rich regions called CpG islands, often found in the promoter regions of these genes (Centelles 2012).

Methylated CpG islands are essentially present in the non-promoter regions of mammalian genomes, while unmethylated CpG islands are commonly located in promoter regions near transcription start sites of normal cells. Genes that contain these unmethylated CpG islands undergo normal transcription in the presence of transcriptional activators.

The CIMP pathway refers to widespread CpG island methylation within promoter regions of tumour suppressor genes. In cancer cells, hypermethylation of CpG islands within these promoter regions leads to transcriptional silencing of tumour suppressor genes and loss of gene function, contributing to the tumorigenic process and commonly silenced genes in CRC patients include *p16*, *p14*, *MGMT* and *hMLH1* (Thomas and Shi 2017). CIMP-positive colorectal tumours are very commonly observed in the proximal colon and show the distinct molecular attributes of poor differentiation, a microsatellite instability status, a higher frequency of *BRAF* mutations than in CIMP-negative tumours, wild-type *KRAS* and a negative association with 18q loss of heterozygosity. CIMP-0 CRC, on the other hand, is known to show 18q LOH positive tumours (Nguyen et al. 2018).

The most common carcinomas arising through CIMP pathway begin with sessile serrated adenoma (SSA), which frequently harbours an activating mutation in the *BRAF* gene. Thus, this pathway has also been called the serrated methylated pathway, a hallmark of the familial serrated polyposis syndrome. SSAs are prone to hypermethylation of a number of genes rich in CpG islands within their promoter regions. Depending on which genes are silenced by hypermethylation, the arising carcinoma may be microsatellite-stable (60% of CIMP1 CRCs) or MSI-H (40% of CIMP1 CRCs). Most sporadic MSI-H CRCs result from epigenetic silencing of *hMLH1* due to hypermethylation of CpG islands in the promoter region. It has been proposed that the loss of hMLH1 protein function in SSAs leads to rapid accumulation of additional mutations in other genes, such as transforming growth factor-β

(*TGFβ*) and *BAX*, which then drive cancer progression. Morphologically, SSAs with *hMLH1* hypermethylation are characterized by cytologic dysplasia, which is followed by the rapid development of malignant transformation. CpG island hypermethylation may also occur in tumour suppressor genes other than *hMLH1*, resulting in CIMP1 MS-S CRCs (Thomas and Shi 2017).

MicroRNAs (miRNAs) have been shown to act as oncogenes or tumour suppressing genes in cancer and are subject to epigenetic silencing through the CIMP pathway (Centelles 2012). In some cases, the presence of epigenetic silencing is overlapped with MSI. Some sporadic CRC with microsatellite instability is caused by DNA methylation. For example, DNA methylation of *MLH1* gene promoter blocks its expression and destroys the ability of MMR system (Tsang et al. 2014).

7.8 Methods of Molecular Testing in CRC

While the revised Bethesda criteria aids in the clinical diagnosis of HNPCC, there have been cases where the diagnosis has been missed. Thus, the National Comprehensive Network NCCN Guidelines recommend HNPCC screening in all individuals with CRC or those diagnosed with CRC at an age less than 70 years or those who meet the revised Bethesda guidelines at an age of more than or equal to 70 years (Gonzalez et al. 2017). The methods of testing include immunohistochemistry and PCR-MSI testing (Townsend et al. 2016).

Immunohistochemistry is done for the four MMR proteins- MLH1, MSH2, MSH6 AND PMS2. 90% of MMR deficient CRCs show a loss of nuclear staining for these proteins. As given in Fig.7.3., MLH1 and PMS2 losses or MSH2 and MSH6 losses are indicative of defective *MLH1* or *MSH2* genes, whereas the isolated loss of PMS2 or MSH6 highlights impaired *PMS2* or *MSH6* genes respectively (Gonzalez et al. 2017).

Immunohistochemical patterns noted in CRCs include cytokeratin-7 negativity and cytokeratin-20 positivity which are observed in 90% well- and moderately-differentiated adenocarcinomas of the colon. These are also used to differentiate between primary CRCs and metastatic carcinomas. Cytokeratin-7 positivity, VEGF-A expression and lumican is observed in poorly differentiated adenocarcinoma colon. Villin has been noted to be present in colorectal adenocarcinomas and absent in melanomas, sarcomas and lymphomas of the colon. CDX2 and tumour-associated glycoprotein have also been noted as important immunohistochemical patterns in CRCs (Hayat 2009).

However, it is necessary to note that MMR-IHC has a few limitations including the possibility of technical failure and false negative staining results in cases of treated rectal cancers (Samowitz n.d.). In addition, while IHC measures the presence or absence of MMR proteins, the presence of MMR protein expression is not a conclusive measure of MMR function as there can be a loss in the function of MMR proteins without a loss of the protein in the cell. Nonetheless, immunohistochemical techniques do allow the specific identification of defective MMR proteins and this is a major advantage of MMR-IHC.

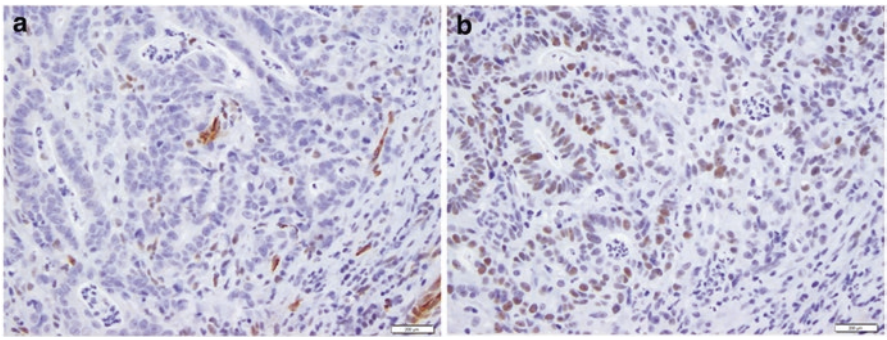

Fig. 7.3 Immunohistochemistry for MLH1 and MSH2. (**a**) Unstained nucleus for MLH1 in the cells of a colorectal adenocarcinoma that also showed the loss of PMS2. (**b**) Intact nuclear expression of MSH2 in the same tumour that also showed intact MSH6 expression. (Gonzalez et al. 2017)

Polymerase chain reaction (PCR)-based MSI testing detects the microsatellite instability in 90% of these tumours. Mononucleotide microsatellite sequences sensitive to transcription errors are used to measure MSI by PCR-amplification with fluorescently labelled primers in fluorescence-based PCR assays. A comparison is made between the amplified fragments and the matched normal fragments. These amplified fragments are then separated by size via capillary electrophoresis and separated by colour of the fluorescent tags on fluorescent labelling. Many different fragments of the same size can be detected in a single reaction and changes in their sizes indicate presence of microsatellite instability, thus making these MSI-High tumours. Instability in two or more markers indicates an MSI-High (MSI-H) status and an MSI-Low (MSI-L) status is interpreted when only one marker is unstable, while a microsatellite stable (MS-S) status exists when all markers are stable (Fig.7.4) (Gonzalez et al. 2017).

This method is considered advantageous as it is also applicable in cases of treated CRCs and allows easy interpretation of results. Since MSI by PCR directly measures changes in DNA due to loss of MMR protein function without measuring the protein themselves, it is also considered a better functional measure of mismatch repair deficiencies.

A combination of the two methods of testing provides added efficacy. A tumour should be tested for *BRAF* mutations and/or MLH1 promoter methylation if it is MSI-H and shows loss of *MLH1* and *PMS2*. In CRCs with loss of MSH2, *EPCAM* deletions must be tested for. Germline genetic testing can also be done using DNA extracted from blood specimens. Methods include

1. Sanger sequencing or next-generation sequencing is used to sequence all coding exons and intron/exon boundaries of the respective MMR genes. A large number of genetic alterations can be analysed simultaneously by NGS which makes it a very lucrative tool. For this purpose, testing of the metastatic lesion is preferred over the primary lesion, especially for synchronous metastatic CRC.

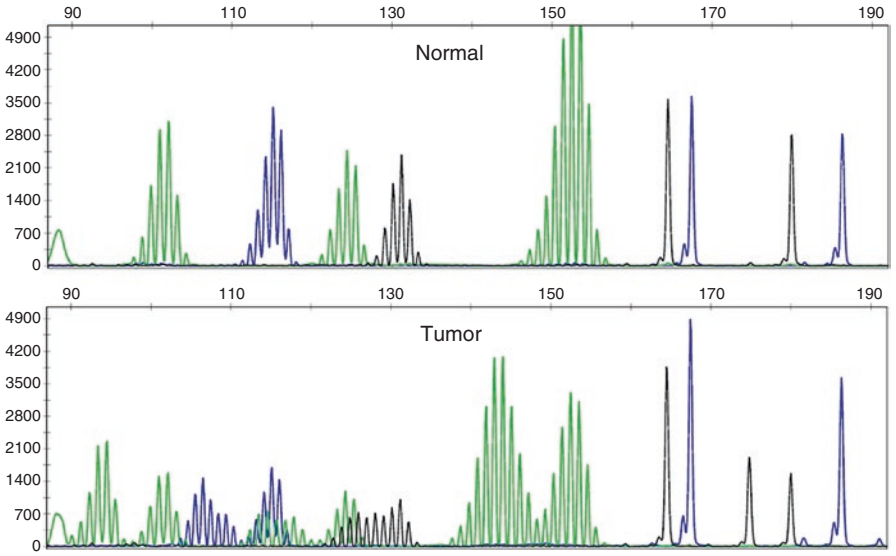

Fig. 7.4 Microsatellite instability is shown in 5 of 5 mononucleotide markers in a PCR-based MSI test for a colorectal carcinoma, comparable with normal, non-cancerous tissue from the same individual. (Gonzalez et al. 2017)

2. Multiplex ligation-dependent probe amplification (MLPA) is a technique that can detect large deletions and arrangements, especially when these MMR gene mutations are not detected by sequencing methods.
3. Southern blot hybridization.
4. Multiplex amplification probe hybridization.
5. Quantitative PCR (qPCR) analysis.
6. Gene-targeted array-based comparative genomic hybridization.

FAP and AFAP- *APC* mutations can be detected by

1. Sanger sequencing.
2. Next-generation sequencing for hereditary CRC is considered cost effective and also requires lesser time as compared to Sanger sequencing (Simbolo et al. 2015).
3. Large rearrangement analysis of the gene.

MUTYH-associated polyposis requires testing of germline *MUTYH* mutations, especially in Caucasians, which is done by (Hegde 2014)

1. PCR/RE digestion-based techniques.
2. Denaturing High Pressure Liquid Chromatography (denaturing HPLC).
3. Pyrosequencing.
4. Sanger sequencing as well next generation sequencing.
5. Allele-specific PCR.

Polymerase Proofreading-associated polyposis is caused due to missense mutations of the exonuclease domains of *POLE* and *POLD1* genes. Familial or early onset MMR-proficient CRC, *APC*-negative and *MUTYH*-negative polyposis can be screened by

1. Sanger sequencing.
2. Next Generation sequencing.

Hamartomatous polyposis syndromes commonly show mutations in *STK11* gene which are identified by

1. Sanger sequencing of the splicing sites.
2. Gene sequence analysis of the entire coding regions.
3. Gross deletion or duplication analysis of the *STK11* gene by MLPA.
4. Juvenile Polyposis Syndrome requires sequence analysis of *BMPR1A* and *SMAD4* genes which is then followed by analysis of these genes for gross deletions or duplications.
5. When no alterations are identified in these genes, mutational analysis of *p10* is done.

In metastatic CRC patients, epidermal growth factor receptor EGFR pathway plays a role in advanced CRCs. While EGFR positivity in tumours on IHC staining does not correlate with treatment response but *KRAS* and *BRAF* mutational status does as these may exist in either a wildtype or mutated state. Thus, testing for mutation in *NRAS*, *KRAS* and *BRAF* is recommended at the time of metastatic diagnosis. *BRAF* mutations are associated with an aggressive form of the disease, shortened progression-free intervals and reduced survival. These mutations are tested by

1. Sanger sequencing
2. Allelic-specific PCR
3. Pyrosequencing to detect point mutations.
4. Multigene assays like the SNapShot platform which combines multiplex PCR amplification of tumour DNA and capillary electrophoresis for the single base extension of the amplified product.
5. Next Generation Sequencing.
6. Fluorescent in situ hybridization has helped detect the expression of VEGF-A mRNA and lumican mRNA in poorly differentiated adenocarcinoma colon with neuroendocrine cell differentiation (Townsend et al. 2016).

7.9 Clinical Implications of Molecular Biomarker Testing in CRCs

- MSI-H cancers have a good prognosis.
- Post-operative 5-FU based adjunctive chemotherapy is ineffective and hence, not recommended for patients with stage II MSI-H CRC.

- MSI testing or MMR-IHC is recommended in all patients with stage II CRC.
- MSI-H tumours better respond to immunotherapy, such as anti-PD1 agents.
- MSI and MMR testing is recommended for all patients with metastatic CRC.
- Commercially available microarray gene expression profiling (Oncotype DX, Genomic Health Inc.) is used to generate a recurrent score in patients with stage two CRC that classifies them according to the risk of recurrence- low risk (score less than 30, recurrence risk 12%), intermediate risk (score 30–40, risk 18%) and high risk (score ≥41, risk 22%).
- Targeted therapies which are directed at the processes involved in tumour growth have gained increased importance such as Anti-VEGF therapies
- Bevacizumab, a recombinant humanized anti-vascular endothelial cell growth factor monoclonal antibody, has been FDA approved for the treatment of patients with advanced CRC in combination with any intravenous 5-FU based regimen (Strickler and Hurwitz 2012).
- Ziv-aflibercept, a fully humanized recombinant fusion protein which blocks angiogenesis by binding to VEGFA, VEGFB and placental growth factor has been FDA approved as a second-line treatment in combination with 5-fluorouracil, leucovorin, and irinotecan (FOLFIRI) in metastatic CRC and the results have been found to be statistically superior to FOLFIRI alone (Sun and Patel 2013).
- Regorafenib, the first and currently only approved oral multi-kinase inhibitor for metastatic CRC blocks several kinases involved in angiogenic and oncogenic survival pathways (VEGFR1, VEGFR2, VEGFR3, TIE2, KIT, RET, RAF1, BRAF, PDGFR, FGFR). It has shown an improved median Overall Survival (Sekhon et al. 2017).
- Targeted anti-epidermal growth factor therapy
  Cetuximab, a chimerized IgG1 antibody that prevents ligand-binding to EGFR and its heterodimers through competitive displacement and Panitumumab, a fully humanized IgG2 antibody targeting EGFR, are FDA approved for patients with metastatic CRC. Cetuximab is also approved as a first line metastatic treatment for patients with wildtype *KRAS* tumours. However, mutated *KRAS* tumours are associated with a decrease in Overall Survival and response rates, particularly with Cetuximab addition, confirming that this mutation is a negative predictor of response to EGFR inhibition (Duff and George 2015).

7.10 Conclusion

With the advent of molecular testing, a new window has been opened in the understanding of the pathophysiology of CRCs and their diagnostic, therapeutic and prognostic realms. It enables a personalized approach to this fatal disease and highlights the importance of secondary prevention in non-communicable diseases as well. With more detailed research in this field, one will also come across novel molecular targets and genetic syndromes of CRC.

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Chapter 8
Nanotechnology Approaches for Colorectal Cancer Diagnosis and Therapy

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Abstract Colorectal cancer (CRC) is a global health problem with limited therapeutic opportunities. It is the most common cancer death in the world, mainly due to its higher incidence and metastatic actions. The molecules expressed by the cancer tissues help to develop several novel approaches in cancer treatment. Nowadys, nanotechnology offers an advanced method for the identification and colon cancer therapy which are capable of overcoming biophysical and biochemical barriers in the body. The studies reveal a hope among the scientific community for developing innovative nanoparticles that possess high adaptability for both diagnosis and therapy. It is a most promising area in therapeutics with different design and formulations for the initiation of controlled drug release and uptake into colon cancer tissue. Different nanoparticles like liposomes, carbon nanotubes, nanoshells, polymeric nanoparticles, and dendrimers have been developed to transport a variety of antitumor agents including siRNA, chemo-modulators, and antiangiogenic mediators. Nanotechnology can significantly expand the precision of targeted drug delivery and helps to reduce the toxic side effects. It can also manipulate the interactions in the gut microbiome and the tumor environment which provide an innovative strategy for cancer treatment. This chapter focuses on the different roles of these nanoparticulate technologies and the potential use of nanoparticle formulations in colon cancer therapeutics. Even though several limitations are hindering in the development of nanotechnologies to function as nanotheranostic mediators, which is expected to pave the way for the fight against colon cancer malignancy.

Keywords Colon cancer · Nanotechnology · Cancer therapy · Nanoparticles · Targeted therapy

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Abbreviations

5-FU	5-Fluorouracil
AuNPs	Gold nanoparticles
CDDS	Colon-specific drug delivery system
CNTs	Carbon nanotubes
CRC	Colorectal cancer
EPR	Enhanced permeability and retention
ESC	Embryonic stem cells
FDA	Food and Drug Administration
GG	Guar gum
GIT	Gastrointestinal tract
HA	Hyaluronic acid
IBD	Inflammatory bowel disease
LP	Lycopene
MDR	Multidrug resistance
MSN	Mesoporous silica nanoparticle
MWCNT	Multi-walled carbon nanotubes
MWNTs	Multi-walled nanotubes
NPs	Nanoparticles
PAMAM	Polyamidoamine
PAX	Paclitaxel
PCL	Polycaprolactone
PEG	Polyethylene glycol
PLA	Polylactide
PLGA	Poly(lactic-co-glycolic acid)
PLK-1	Polo-like kinase 1
RCT	Randomized controlled trial
ROS	Reactive oxygen species
SWNTs	Single-walled nanotubes
US FDA	United States Food and Drug Administration
XG	Xanthan gum

8.1 Introduction

Colon cancer, also mentioned as colorectal cancer (CRC), is developed by the uncontrolled growth of tumor cells in the cecum, colon, or rectum. CRC is the one of the major causes of cancer mortality worldwide with approximately 1.4 million cases. It is recognized as the third most and second most cancer in men and women of around 10% and 9.2% of the entire worldwide cases, respectively (Ferlay et al. 2015). Compared to other malignancies, metastasis is the major cause of CRC . The primary site of CRC metastasis is mostly developed in the liver (Siegel et al. 2014).

Therefore, the early stage of diagnosis helps in higher chance of survival rate. The CRC onset is mainly due to intrinsic features, such as gender, aging, and genetic factors. Chronic diseased conditions such as obesity, inflammatory bowel disease (IBD), and diabetes are also related with higher risk of colon cancer (Ma et al. 2013; Tsilidis et al. 2015). Gut microbial imbalance can also trigger inflammation, reactive oxygen species (ROS) production, and microbial metabolism products (toxins) that can promote genetic modifications leading to colon cancer. Lifestyle-associated factors, such as smoking, alcohol consumption, and high-fat and high processed meat intake, also have been connected to polyp growth and CRC development (Cross et al. 2010; Chen et al. 2015). Presently, various therapies are available for CRC, comprising chemotherapy, surgery, and radiation therapy. In late stages, chemotherapy is the main treatment line to reduce CRC progression.

Even though chemotherapy is the most common approach for cancer treatments, using individual chemotherapeutic agents results in a range of side effects that are still a major concern in chemotherapy. In conventional chemotherapy, 5-fluorouracil is a commom chemotherapeutic drug used in CRC along with some other drugs such as leucovorin, oxaliplatin and irinotecan. (Mazhar et al. 2006). Other than drugs, monoclonal antibodies like cetuximab are also recognized for CRC treatment (Van Cutsem et al. 2009). Chemotherapy has its own limitations, such as low circulation time, instability, lack of target specificity, and chance of overdosage; all these factors lead to toxicity and more side effects. Recently, there has been a higher interest in the field of nanotechnology that has shown a constant and target-specific delivery by using nanosized delivery systems which may pave a way for the novel ways toward effective and safe targeted colon therapies.

Nanotechnology as an emerging tool for colon targeted cancer therapy, which has received more interest and express significant therapeutic benefits. Nanotechnology can be a novel effective therapeutic strategy, which can overcome various challenges related with colon cancer therapy and drug development (Arshad et al. 2020). Various types of nanomaterials are developed and being investigated that could show improved cellular uptake, target specificity, and extended circulation for cancer treatment. In addition, their high surface area/volume ratio helps to incorporate several therapeutic drugs that are distributed to cancer tissues through EPR effect after entering into the leaky tumor vasculatures (Torchilin 2011; Prabhakar et al. 2013). Because of these characteristic features, nanomaterial-based therapeutics for cancer have shown similar or even better anticancer effect to marketable formulations, by exhibiting decreased side effects and providing new approaches to fight against colon cancer.

The advantage of nanosized carriers is that they can increase drug therapeutic index either encapsulated or conjugated to the nanocarrier's surface. Size is a key factor in the delivery of nanotechnology-based cancer therapeutics (Fig. 8.1). Nanotherapeutic delivery primarily focuses on enhanced permeability and retention (EPR) effect by passive targeting on tumor tissue. This phenomenon is specific to tumor microenvironment, along with enlarged tumor vasculature permeability, which allows nanoparticles (<200 nm) to enter and accumulate in the cancer microenvironment. Besides, the site and time of drug release can also be controlled by

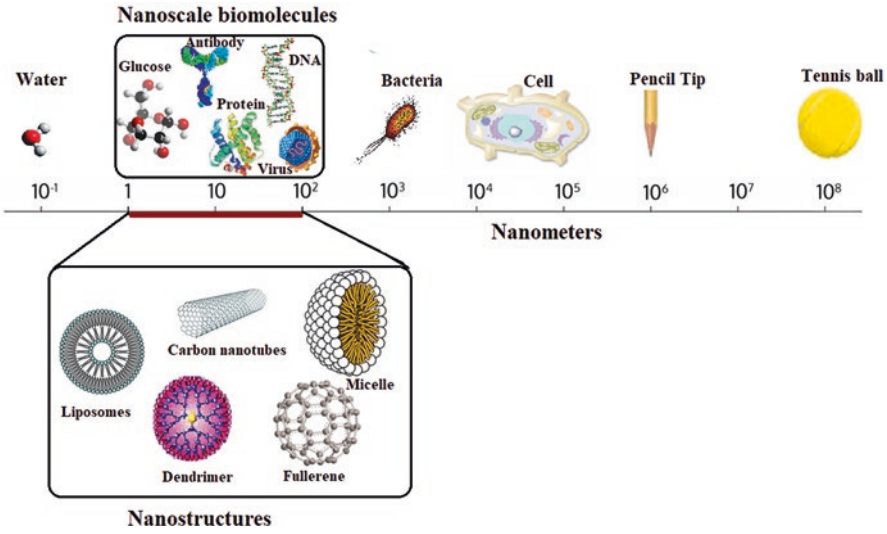

Fig. 8.1 Nanotechnology devices are 100–10,000 times smaller than human cells

stimulated actions, such as pH, ultrasound, material composition, or temperature. Although a few numbers of nanosized delivery carriers have been approved for human usage. Nanosized carriers have favorable surface characteristics, which is one of the benefits that enable them to be functionalized at the desired site of action (Torchilin 2011). These nanocarriers show higher efficiency and reduced side effects by lower accumulation rates in normal tissues, with higher accumulation and retention rates in cancer tissues (Prabhakar et al. 2013). It might also protect the drugs from inappropriate environmental conditions and enzymatic actions, as well as against early washout from the body. Moreover, due to their nanosize, it directly interacts with cell membrane and intracellular structures. The nanotechnology application has therefore provided a huge capability in overcoming intracellular mechanisms linked with drug resistance. Nanoparticles (NPs) have an important role in reversing multidrug resistance (MDR) by permitting sufficient drug to persist in the cytoplasm in both pump-dependent and pump-independent MDR. Nanocarriers induced enormous intracellular drug loading capacity, which overcomes many limitation progressions related to intake, metabolism, and efflux of drug, and noticeably increase the efficacy of chemotherapy.

8.2 Nanomedicines/Nanodevices for Colorectal Cancer Treatment

Nanomedicine can be delivered by oral, inhalation, rectal, or systemic route. Administration through oral route appears to be mostly improved and acceptable in the gastrointestinal disease treatment for patient's comfort (Homayun et al. 2019).

Table 8.1 Types of nanomaterials used for colon cancer therapies (Prabhakar et al. 2013; Masood 2016)

Material type	Nanostructures	Size	Main component
Organic	Polymeric NPs	10–1000 nm	Polymer
	Liposomes	10–1000 nm	Lipid
	Dendrimers	1–15 nm	Polyamidoamine
	Polymeric micelles	20–200 nm	Polymer
Inorganic	Carbon nanotubes	0.4–100 nm	Carbon
	Fullerenes	0.4–1.3 nm	Carbon
	Nanoshells	1–20 nm	Silica
	Silica NPs	20–100 nm	Silica

The major challenge in oral intake for colon-specific drug delivery system (CDDS) is the protection of drug against its release and absorption from gastrointestinal tract (GIT) to the colon. Also, it should not be degraded in GIT sites and only released and absorbed at the colon-specific site (Banerjee and Mitragotri 2017). Nanotechnological studies mainly concentrate on inorganic nanomaterials, such as carbon nanotubes (CNTs), silica nanoparticles (NPs), fullerenes, and nanoshells, and organic nanomaterials, such as liposomes, polymeric nanoparticles, dendrimers, polymeric micelles, etc. (Table 8.1). Nanoparticles also offer various CRC treatment strategies combined with chemotherapeutic drugs, including 5-fluorouracil (5-FU) and irinotecan, as well as targeted therapies using antibodies (cetuximab, rapamycin). The therapeutic approaches of nanoparticles through photosensitizing or thermal destruction of malignant cells via intratumoral release can also be an innovative approach for tumor cell destruction.

8.2.1 Organic Nanomaterials for Colon Cancer Therapies

Organic polymers possess very good properties such as biocompatibility and degradability. Organic-based nanomaterials from natural or synthetic polymer have been widely used in the field of CRC therapies nowadays. The organic nanostructures can be divided into polymeric nanoparticles (NPs), liposomes, dendrimers, polymeric micelles, hydrogel, and nanoemulsion.

8.2.1.1 Polymeric Nanoparticles

In the field of nanomedicine, polymeric NPs possess greater pharmacokinetics, drug loading capability, and stability than polymeric micelles. Polymeric matrix comprises media for the drug absorption, entrapment, and encapsulation (Masood 2016; Devulapally and Paulmurugan 2014). Polymeric nanoparticle structures range in size between 10 and 100 nm. The outer surface of polymeric NPs comprises of

nonionic surfactants, which helps to minimize immunological interactions (Torchilin 2008; Bilensoy et al. 2009). The two main polymeric NPs that have been permitted by US FDA are poly(lactic-co-glycolic acid) (PLGA) and polycaprolactone (PCL) (Zhang et al. 2007).

5-Fluorouracil, which is employed in the initial phase for CRC therapy, also affects the healthy cells, and the bioavailability is also not promising in the colon region. Several studies stated that the 5-FU delivery can cause harmful general side effects, such as diarrhea, mucositis, changes in gut microflora, and bacterial translocations. Jain et al. have developed hyaluronic acid (HA)-coupled chitosan nanoparticles (HACTNP) incorporated with 5-fluorouracil (5-FU) and oxaliplatin for effective colon cancer delivery system (Jain and Jain 2008; Jain 2010). It shows a significant uptake by HT-29, colonic cancer cells, and higher cellular toxicity as compared to the 5-FU solution. In another study, 5-FU-incorporated nanoparticles with citrus pectin and pH-responsive enteric polymer (Eudragit S100) were used as targeted drug delivery systems for colon cancer (Subudhi et al. 2015). They concluded that polymer pectin was a promising carrier for the development of colon-specific delivery system. The toxicity and efficacy of Eudragit S100-coated CPN-loaded 5-FU in CRC were also studied in both cellular and animal models (Subudhi et al. 2015; Tummala et al. 2015). Eudragit S100 is a pH-responsive enteric polymer that does not degrade below pH 7 and is mainly used for coating nanoparticles. The main advantage of Eudragit S100 coating was to avoid drug release in gastrointestinal tract and only release at targeted colon site (Obeidat and Price 2006; Asghar and Chandran 2008). 5-FU may also exhibit toxicity on the gut bacteria that are involved in dietary fiber metabolism including polysaccharides such as chitosan, xanthan gum (XG), guar gum (GG), and amylose. Site-targeted delivery of 5-FU carrier was coated with natural gums such as GG and XG, which permit drug release in the colon and also provide nutrients for probiotics, thereby enabling gut homeostasis. On the other hand, the coadministration of probiotics can overcome the 5-FU nanoparticle-linked side effects on the colon bacteria (Singh et al. 2015).

8.2.1.2 Liposomes

Liposomes are first nanocarriers introduced by Bangham et al. in 1961 (Bangham et al. 1965). Liposomes are the first drug delivery system approved by FDA for clinical purposes (Pattni et al. 2015). Nanoliposomes are tiny, spherical nontoxic carriers enclosing an aqueous core with phospholipid bilayer and cholesterol. Liposomes are promising drug delivery systems for cells, due to their size, ability to incorporate numerous substances, slow-releasing capability, targeted delivery, and reduced side effects (Suntres 2011; Patil and Jadhav 2014). Nanoliposomes are most commonly used nanoparticles for delivering proteins, small peptides, and nucleic acids. In cancer treatment, to enhance the site-specific delivery of drugs, cell-specific receptors and ligands are inserted within a lipid bilayer. Because of their amphiphilic nature, nanoliposomes are also used for site-specific delivery of hydrophobic anticancer drugs (Andresen et al. 2005; Huynh et al. 2009; Abreu et al. 2011).

Nanoliposomes are mainly divided into the following:

I. Stealth liposomes or long-circulating liposomes: Here, the liposome bilayer is reformed by adding polyethylene glycol (PEG) or gangliosides, which aid to avoid the binding of plasma opsonins to the surface of liposome and allow it to remain stable in the circulation (Nag and Awasthi 2013; Noble et al. 2014).
II. Active nanoliposomes: Liposome nanoparticle targets antibodies, peptides, glycoside residues, receptors, and hormones.
III. Sensitive nanoliposomes: Liposomes with specific properties such as pH-sensitive, thermosensitive, and magnetic (Akbarzadeh et al. 2013).

Doxorubicin (Doxil) and (DaunoXome), anticancer drugs loaded with liposome, have been approved by FDA for CRC (Barenholz 2012). Another nanoliposomal drug is ThermoDox (thermosensitive liposome doxorubicin) that targets colon liver metastasis in combination with radiofrequency. This nanoliposomal formulation releases the drug upon a slight hyperthermic trigger. ThermoDox has shown 25-fold more site-specific delivery of doxorubicin than IV doxorubicin (Stang et al. 2012). In addition to this liposome, nanocarriers, viz., CPX1, LE-SN38, and CPX-1 (irinotecan HCl: floxuridine), have cleared phase II trials for the treatment of CRC (Patel 2008; Loira-Pastoriza et al. 2014; Jefremow and Neurath 2020). Magnetoliposomes show many interesting properties, like magnetically directed drug delivery, hyperthermia-induced drug delivery, and high drug loading efficiency. To improve the antitumor activity against CRC in rat model, a monoclonal antibody-coupled liposomal 5-fluoro-2′-deoxyuridine nanocarrier is developed (Koning et al. 2002). A cationic nanoliposome is used to investigate the therapeutic effect of double gene therapy combined with immunotherapy for tumor cells. It has significant result in improving the survival rate and quality of life of CRC in mouse model (Sun et al. 2012). Handali et al. developed a novel folic acid-conjugated liposome for the targeted delivery of 5-fluorouracil to colorectal cancer cells (Handali et al. 2018). It might increase the therapeutic efficacy of drug while reducing the toxic side effects. One of the main challenges in the treatment of CRC is to overcome the multidrug resistance (MDR) protein-induced tumor resistance. A liposomal formulation developed with hyaluronic acid coating is found to be very efficient for the delivery of drug imatinib mesylate against MDR colorectal cancer (Negi et al. 2015). Several liposome-based nanosystems are under preclinical development, liposomal curcumin, liposomes (PEG-liposomal L-OHP), etc. (Patel 2008; Cay et al. 1997).

8.2.1.3 Dendrimers

Dendrimers are monodisperse molecules characterized with an extremely branched 3D structure (Bharali et al. 2009; Somani and Dufès 2014). They are highly water soluble due to the presence of hydrophilic functional groups (Nanjwade et al. 2009). Dendrimers can load drugs as well as gene through covalent conjugations and electrostatic interactions. They consist of vacant inner cavities and a bulk of surface functional groups, which possess a promising carrier for anticancer therapy. Due to

comparatively small size (1–15 nm), dendritic carriers can be easily cleared off from the body through kidneys, thereby reducing in vivo toxicity (Padilla De Jesús et al. 2002). Among all the dendrimers, the most broadly studied dendrimer is polyamidoamine (PAMAM), and its surface is covered with a huge number of amine groups, which helps to conjugate with other functional moieties. It is one of the most sophisticated nanotechnology devices for targeted drug delivery (Semwal et al. 2010). Tomalia et al. described first PAMAM dendrimers in 1985 (Tomalia et al. 1985). Due to their extremely branched structure, dendrimers have open spaces between branches, so they can easily encapsulate drugs (Bhadra et al. 2003). Mignani et al. have demonstrated DOX dendrimer was 10 times less toxic than DOX alone solution after exposure to colon carcinoma cells for 72 h. Administration of DOX dendrimer to BALB/c mice bearing colon carcinoma tumors also resulted in higher uptake than plain DOX at 48 h (Mignani and Majoral 2013).

8.2.1.4 Polymeric Micelles (PMs)

Polymeric micelles (PMs) are nanosized particles with both hydrophilic shell and hydrophobic core (Torchilin 2007; Wu et al. 2013a). The hydrophilic shell of micelle is commonly constructed with PEG, which helps to stabilize and protect the carriers from degradation in vivo *(*Kataoka et al. 2012*;* Zhou et al. 2017*)*. For hydrophobic core construction, several natural as well as synthetic polymers have been commonly used, including polysaccharides, PCL, PLA, and PLGA. The core provides a better medium to encapsulate hydrophobic drugs, thereby solving their poor water solubility. Oxaliplatin NPs were made up of chitosan oligosaccharide and stearic acid polymer, which helps in polymeric micelle development. These micelles exhibited good internalization capacity and higher oxaliplatin accumulation in tissues. Moreover, it also showed higher cytotoxicity against most of the tumor cells when compared to oxaliplatin alone (Wang et al. 2011). Another targeted therapy of CRC was developed using a TORC1 signaling complex inhibitor called rapamycin in pegylated octadecyl lithocholate micelles linked with LTTHYKL peptide against CRC. These micelles showed a higher beneficial efficacy than free rapamycin drug in mice model, with significantly lower toxicity (Khondee et al. 2015). Another predictive factor PLK1 is considered to classify patients expected to respond to randomized controlled trial (RCT) (Rödel et al. 2010; Fernandez-Acenero et al. 2016). Nowadays, several inhibitors are targeting PLK for the delivery system development mixture mode.

8.2.2 Inorganic Nanomaterials for Colon Cancer Therapies

In addition to organic NPs, several other inorganic materials with exciting confirmation as well as exclusive chemico-physical properties have also been identified as NPs for colon cancer therapies. Some of these are discussed below.

8.2.2.1 Carbon Nanotubes

Carbon nanotubes (CNTs) are allotropes of carbon with a cylindrical-shaped nanostructure (Wang et al. 2009). CNTs have unique properties that include its improved geometrical, mechanical, and electrical properties, stiffness, thermal conductivity, strength, etc. These unique properties are exploited in the field of nanotechnology, electronics, optics, material science, and technology (Gullapalli and Wong 2011; Lim et al. 2014). Based on their structure, CNTs are classified into single-walled nanotubes (SWNTs), consisting of a single graphite sheet covered into a cylindrical tube, and multi-walled nanotubes (MWNTs) comprising group of nanotubes concentrically nested together as the rings of a tree trunk. Functionalized CNTs are used as novel nanocarriers for proteins, drugs, and genes (Ghorbani and Karimi 2015). The anticancer activity of paclitaxel (PAX)-loaded nanotubes and its cellular interactions were studied using poly(2-(dimethylamino)ethyl methacrylate-co-methacrylic acid) on MC38 murine colon cell line. It has shown effective anticancer action against CRC (Lee and Geckeler 2012). MWCNT (Multi-walled carbon nanotubes) is combined with embryonic stem cells (ESC) as cellular agents exhibiting anticancer activity against MC38 cancer cells (Mocan and Iancu 2011). MWCNT conjugated with folic acid is used as nanosystem for the delivery of photothermal drug against CRC and the drug-targeting efficiency improved by folic acid conjugation (Wen et al. 2013). MWCNT conjugated with hyaluronic acid and PEG shows sustained gemcitabine (GEM) release against colon adenocarcinoma cell lines (HT-29), and the side effects of GEM can be reduced as a result of hydrolysis within cancer cells (Prajapati et al. 2019). MWCNTs loaded with oxaliplatin also confirmed effective drug release against HT-29 cells (Wu et al. 2013b). In addition to MWCNTs, SWCNT modified with antibody C225 can be an active nanosystem for specific delivery of antitumor drug 7-ethyl-10-hydroxy-camptothecin (SN38) into EGFR-expressing colorectal cancer cells (Lee et al. 2013). CNTs are one of the promising nanocarriers used in the treatment and diagnosis of various types of cancer. Many studies have been carried out on CNTs as nanosystems; not much work is reported on colorectal cancer. Moreover, further research is required to show any disadvantages or side effects caused by CNTs.

8.2.2.2 Nanoshells

Nanoshells are spherical nanoparticles containing a dielectric core which is surrounded by a shell (Fuchigami et al. 2012). These nanoparticles can be designed to have unique chemical and optical properties by varying the geometry, such that they can be useful for biological applications. Nanoshell materials can be made from metals, semiconductors, or insulators. Gold nanoshells are metal nanoshells, which are mainly used for in vitro cancer detection, imaging, and treatment (Mody et al. 2010). Nanoshells are used for site-specific delivery of certain anticancer drugs like paclitaxel, doxorubicin, and siRNA. These are coated with PEG or other functional groups, which enhance the efficacy of delivery and bioavailability (Mudshinge et al.

2011). For the treatment of colon cancer cells, plasmonic photothermal therapy using gold nanoshell exhibited promising results (Koohi et al. 2017). Platinum drug-loaded gold nanoshells are used to study the combined effect of chemo-photothermal therapy in CRC. Poly[2-(N,N-dimethylamino)ethyl methacrylate]-poly(ε-caprolactone) micellar template-based gold nanoshell has been used as a nanosystem for the delivery of platinum-based drug, dichloro(1,2-diaminocyclohexane)platinum(II) (DACHPt) (Lee and Shieh 2020). This DACHPt-loaded nanoshell showed combined chemo-photothermal therapy which results in tumor growth inhibition with less side effects (Lee and Shieh 2020). Guanylyl cyclase C is targeted by bacterial heat-stable enterotoxin conjugated gold nanoshell, which helps to induce thermal abalation in metastatic colorectal cancer by heat release during excitation by using near-infrared (NIR) light exposure (Waldman et al. 2006).

8.2.2.3 Fullerene Derivatives

Fullerenes are carbon-based compounds made in the form of hollow sphere, tube, or ellipsoid. The most stable, common, and high symmetry fullerene is C60. C70, C20, carbon nano-onions, nanobuds, and CNs (elongated, tube-structured fullerene) are other fullerene variants (Nasibulin et al. 2007). Their size, three-dimensionality, hydrophobicity, electronic configurations, and photoexcitation make them an interesting topic in various medical fields. C60 is insoluble and aggregates easily in aqueous media. Generally, carriers like calixarenes, cyclodextrins, polyvinylpyrrolidone, liposomes, and micelles are used for fullerenes encapsulation. Chemical functionalization can increase the hydrophilicity mainly done with carboxylic acid, amino acid, amphiphilic polymers, and polyhydroxyl group. Fullerene can be used as antioxidant and radical scavenger (Caruso et al. 2014). Moreover, for drug and gene delivery, fullerenes have been used as a nanocarrier. In vitro studies with C60 fullerene derivatives decrease the migration and invasion of HT-29 CRC cells (Lucafo et al. 2014). Fullerenes are used as a photosensitizer to mediate intraperitoneal photodynamic therapy for abdominal dissemination of colon adenocarcinoma in mouse model. Fullerenol shows protective effects on cardiotoxicity and hepatotoxicity caused by doxorubicin in rats with CRC (Injac et al. 2009). The C60 fullerenol nanoparticles (FNP) efficiently inhibited the development of dysplastic aberrant crypt foci in dimethylhydrazine-induced rat model of CRC (Perše et al. 2011). The antineoplastic activity of 5-fluorouracil (5-FU) and pyrrole derivative 1-(4-Cl-benzyl)-3-Cl-4-(CF3-fenylamino)-1H-pyrrol-2.5-dione (MI-1) are compared with pristine C60 fullerene (C60FAS) in rat model which induced CRC by 1,2-dimethylhydrazine (DHM). It is found that when rats are treated with C60FAS, 5-FU, and MI-1, the number of tumors and lesion area is reduced and it also decreases the side effects of antitumor therapy (Lynchak et al. 2017).

8.2.2.4 Silica Nanoparticles

Silica nanoparticles exhibit a porous framework with several benefits including higher biocompatibility and functionalization (Amato 2010; Wei et al. 2010). These nanoparticles are like a porous construction like a beehive, which is able to entrap large quantities of various bioactive components. Important features of mesoporous silica nanoparticles include adjustable size range of 50–300 and cavities of 2–6 nm (Stang et al. 2012). And it also shows lower toxicity, easy endocytosis, and resistance against external factors like temperature and pH (Bharti et al. 2015). Radhakrishnan et al. developed mesoporous silica nanoparticle (MSN) with protamine hybrid system to control drug release in cancer cells where definite enzymes can activate the drug activity (Radhakrishnan et al. 2014). This study also significantly enhanced the cell death in comparison to the free drug with MSN-PRM system. Cellular uptake was improved in DOX-loaded HA-MSNs, and it is also shown that DOX-HA-MSNs exhibit more cytotoxicity to HCT-116 cell lines than free DOX drug (Gidding et al. 1999).

8.3 Conclusions

In this chapter, different types of nanodevices were discussed including organic (polymeric NPs, liposomes, dendrimers, polymeric micelles) and inorganic (carbon nanotubes, nanorods, fullerenes) nanoparticles. These devices are developed by improving their structural and cellular targeting abilities, which promoted more effective therapeutic delivery. Of all the nanomaterials presented in this chapter, liposomes are highly advanced and are clinically permitted for clinical trials with some formulations already available in the market, while other inorganic-based formulations have not received such approval. Though some nanomaterial formations are already approved for various cancer therapies, e.g., Abraxane, Doxil, and Embosphere., successful clinical studies are limited, possibly due to the inconsistency of patients and different tumor pathological characteristics, opsonization, pharmacokinetics, tumor accessibility, as well as biodistribution. Certainly, almost all therapeutic methods are depending on EPR effect which intensely differs from one patient to other and from tumor to tumor.

Regardless of outstanding health benefits, the effective conversion of nanomaterial therapeutics into medical practice still has many concerns and challenges including pharmacokinetics and targeting efficacy. In the near future, scientifically validated nanomaterials for medical usage will be developed. The important restriction which delays the transformation of nanodevices to bedside is the difficulties to meet drug regulatory approval for the design and scaling up of nanodevices. The development of new nanodevices for colon cancer treatment is probably another way to have more innovative clinical trials in the future.

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Chapter 9
Natural Products as Immunomodulatory and Chemosensitizing Agents in Colon Cancer Treatment

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Abstract Colorectal cancer is a common public health problem and is the leading cause of mortality and morbidity across the globe. Epidemiological, preclinical, and clinical studies have highlighted the critical relationship between the immune system and colon carcinogenesis. This has led to use of immunomodulatory therapy in adjuvant or palliative treatment of colon cancer. Natural products found in fruits, vegetables, and dietary spices have been demonstrated to exhibit protective effects against development of various human diseases including colon cancer. The anticancer effect of these natural products has been linked to their immunomodulatory and anti-inflammatory activity. Natural products are reported to activate a subset of immune cells, inhibit immune checkpoints, and modulate the production of various cytokines and chemokines, thus overall strengthening the immune surveillance. In this chapter, we will discuss the recent advancements in immunomodulatory and anti-inflammatory activities of several natural products which eventually inhibit the development of colon cancer. We will also highlight the chemosensitizing potential of these phytochemicals in combination with standard colon cancer therapies.

Keywords Colorectal cancer · Natural products · Phytochemicals · Immunomodulation · Inflammation · Combination therapy

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Abbreviations

5-FU	5-Fluorouracil
ACF	Aberrant crypt foci
BCNU	1,3-Bis(2-chloroethyl)-1-nitrosourea
COX-2	Cyclooxygenase 2
CRC	Colorectal cancer
CTLA-4	Cytotoxic T-lymphocyte-associated protein 4
DMH	1,2-Dimethylhydrazine
DSS	Dextran sulfate sodium
EGCG	Epigallocatechin-3-gallate
EGFR	Epidermal growth factor receptor
EMT pathway	Epithelial-to-mesenchymal transition pathway
HIF1-α	Hypoxia-inducible factor 1-alpha
HPV	Human papillomavirus
ICPIs	Immune checkpoint inhibitors
IDO	Indoleamine-2,3-dioxygenase
IFN-γ	Interferon-gamma
IL-10	Interleukin-10
LAG-3	Lymphocyte activation gene 3
MDR1	Multidrug resistance protein 1
MHC-1	Major histocompatibility complex-1
MSI	Microsatellite instability
NF-κB	Nuclear factor kappa B
NK cells	Natural killer cells
NKG2D-L	Natural killer group 2, member D ligand
Nrf2	Nuclear factor erythroid 2-related factor 2
PARP	Poly(ADP-ribose) polymerase
PD-1	Programmed cell death protein 1
PGE_2	Prostaglandin E2
STAT1	Signal transducer and activator of transcription 1
TGF-β	Transforming growth factor-β
VEGF	Vascular endothelial growth factor

9.1 Introduction

Colorectal cancer (CRC) is the second leading cause of cancer-related deaths worldwide that affects equally men and women (Siegel et al. 2020). There have been an estimated 1,47,950 new CRC cases, and 53,200 deaths are expected in 2020 in the USA alone (Siegel et al. 2020). Additionally, American Cancer Society reports showed that CRC incidence rate is increasing in adults under 50 years (Singh et al. 2014; Fidler et al. 2017). The incidence of colon cancer is thought to be low in Asia

as compared to Western countries. However, recent studies from Korea, Japan, Hong Kong, and China have shown an increasing trend in the incidence rate of CRC in these countries (Deng 2017).

Colon cancer pathogenesis follows the dysplasia-aberrant crypt foci (ACF)-adenoma-carcinoma sequence and involves a series of morphological, histological, and genetic changes that accumulate over years or decades (Rattray et al. 2017). The genetic factors account for only 5–10% of human colon cancer and include familial adenomatous polyposis (1%), hamartomatous polyposis, and Lynch syndrome (2–4%). On the other hand, the majority of colon cancer cases are linked to environmental factors such as diet with high proportion of red meat, alcohol consumption, smoking, and lack of daily physical exercise (Moss and Nalankilli 2017; Thanikachalam and Khan 2019). These risk factors collectively perturb the normal colon physiology and upregulate the expression of various tumor-promoting processes.

Generally, it takes 10–15 years for CRC development which offers a great opportunity for prevention strategies. Epidemiological data have suggested that regular consumption of vegetables, fruits, legumes, and whole grains lowers the risk of developing CRC. People are now preferring common medicines of natural origin over synthetic, keeping in mind that medicines of natural origin are pharmacologically safe and do not produce side effects. The phytochemicals present in these foods suppress proliferation and trigger apoptosis of transformed cells. Based on chemical structure and biological function(s), phytochemicals can be categorized into several groups such as phenolic compounds (flavonoids, phenolic acids, coumarins, tannins, and stilbenes), carotenoids (α,β-carotene, lycopene, lutein), organosulfur compounds (allyl sulfur compounds, isothiocyanates, and indoles), and alkaloids. The inhibitory action of various phytochemicals on cell growth and proliferation, apoptosis, chronic inflammation, angiogenesis, and metastasis has indicated that these molecules can be taken as lead compounds to design novel chemopreventive agents. In this regard, several small molecules of natural origin including curcumin, resveratrol, catechins, lycopene, silymarin, genistein, etc. have been documented to possess potent chemopreventive properties (Chikara et al. 2018). From 1981 to 2019, more than 50% of all approved drugs either were of natural origin or mimicked the natural molecule in one way or the other way (Newman and Cragg 2020).

While most of the CRC prevention studies have mainly focused on intrinsic alterations in cancer cells, the vital role of the immune system in preventing CRC development has recently been appreciated. Paul Ehrlich first proposed the role of the immune system in protecting the host from tumor development. L. Thomas suggested the existence of an immunological surveillance mechanism stating that newly transformed cells are recognized by local immune cells and are eventually eliminated. It was suggested that cancer cells express specific antigens called neo-antigens and therefore are recognized as foreign antigens by the host immune system, which generates an immunologic response for their elimination. Later on, L. Gross experimentally proved this hypothesis by showing the protective role of immunization

against sarcoma (Gross 1943). Sir Burnet has been credited for the formulation of the "immune surveillance theory" (Burnet 1970). A consistent alteration in the tumor-infiltrating immune cell repertoire has been observed in the human CRC initiation, promotion, and progression phase providing heterogeneity to the developing tumor which further underpinned the immune surveillance theory (Galon et al. 2006). Thereafter, several animal model studies established immune surveillance as a primary defense mechanism against CRC development (Budhu et al. 2014). In this regard, immunoprevention – the use of immune-modulating agents such as vaccines for cancer prevention – has emerged as a promising window for cancers caused by viral infection, for example, HPV-induced cervical cancer (Finn and Beatty 2016; Kensler et al. 2016; Spira et al. 2016). However, the applicability of such vaccines for the prevention of nonviral malignancies including CRC is yet to be verified.

The undesired side effects and low response rate of chemotherapeutic agents are the major obstacles of modern-day CRC treatment. In this regard, several natural compounds have been combined with standard chemotherapeutic agents such as 5-FU to chemosensitize tumor cells to conventional therapies. The accumulating evidences have suggested that natural compounds and dietary components modulate immune response to potentiate anticancer effects of therapeutic drugs. This chapter focuses on detailed discussion on immunomodulatory and chemosensitizing properties of well-studied natural compounds including curcumin, resveratrol, and epigallocatechin-3-gallate (EGCG). This chapter also provides recent information on CRC clinical trials conducted to evaluate immunomodulatory and chemosensitizing effects of natural compounds.

9.2 Immune Surveillance: Basic Mechanism and Biological Function

Immune surveillance serves as the principal safeguarding barrier involving recognition, interaction, and elimination of newly arisen cancer cells. However, this interplay between immune cells and tumor cells is much more complex than previously thought. Research over the past few decades has suggested that the immune system plays a dual role in CRC progression: first, activation of cytotoxic T-lymphocytes to kill the neoplastic growth in the body and, second, secretion of cytokines and chemokines of inflammatory origin, thereby fostering tumor cell growth and survival (Ferrone and Dranoff 2010; Gonzalez et al. 2018). Based on these progressive findings, the concept of immune surveillance was refined to immunoediting (Dunn et al. 2002). Immunoediting represents a dynamic process which describes the immunosuppressive aspect of the tumor microenvironment. Immunoediting is governed by three Es: elimination, equilibrium, and escape (Dunn et al. 2004).

9.2.1 Elimination

The elimination phase represents the refined and modern form of the immune surveillance concept, demonstrating the synergistic efforts of innate and adaptive immune systems to eliminate cancer cells. One of the most common features of solid tumors is infiltration of immune cells. How naive immune cells are activated and recognize and employ mechanisms to eliminate developing tumors – the information on this whole mechanism – is poorly understood because these processes occur in vivo and hence remain hypothetical. It has been proposed that tumor cells generate certain specific signals which are sufficient to alert and activate the immune system. For example, the natural killer group 2, member D ligand (NKG2D-L) is shed by tumor cells which triggers the activation of its respective receptor on NK cells for cancer cell elimination (Guerra et al. 2008). Additionally, neo-transformed cells or tumor cells undergoing apoptosis produce "danger signals" that activate effector cells of adaptive and innate immune systems such as cytotoxic T-cells and neutrophils (Behrens et al. 2008). However, till date, the elimination phase has not been visually observed in vivo which could be due to lack of sophisticated tools for capturing these molecular events, i.e., interplay of immune cells and cancer cells during the initiation phase of CRC development.

9.2.2 Equilibrium

Cancer cell variants that have survived the immune surveillance elimination phase enter into the equilibrium phase. While the elimination phase involves exercise of both innate and adaptive immune systems, the equilibrium phase is mainly driven by the adaptive immune system. Immune selection posed during tumor growth produces tumor cell variants with reduced immunogenicity. The main purpose of the equilibrium phase is progressive sculpturing of tumor cells which are resistant to immune effector cells via reducing immunogenicity. These tumor cell variants easily survive in an immunocompetent host. This equilibrium condition with such tumor cell variants also explains the apparent paradox of CRC development in healthy individuals. A lot of research is needed to be done to demonstrate the mechanistic puzzle of the equilibrium phase. Koebel et al.'s research report was a milestone in providing proof of concept of the equilibrium phase (Koebel et al. 2007). It was shown that depletion in T-cell and IFN-γ resulted in spontaneous tumor formation at the site of chemical carcinogen (methylcholanthrene) administration in immunocompetent mice (Shankaran et al. 2001). Further studies in mice have supported the critical role of the immune system in maintaining the equilibrium phase (Loeser et al. 2007; Eyles et al. 2010). As the equilibrium phase is characterized by regular elimination of parental cancer cells and production of new daughter tumor cells with reduced immunogenicity under immunological selection cues, it is the longest phase of the immune editing process.

9.2.3 Escape

Few tumor variants produced in the equilibrium phase survive the intrinsic as well as extrinsic apoptotic signals and enter into the escape phase. There are several mechanisms at the tumor cell level and/or tumor microenvironment level which favor tumor cell escape from death signals. Reduction in tumor cell immunogenicity is the common pathway followed by lowering immune recognition. Loss of the MHC-1 protein has commonly been observed in multiple cancers including CRC (Campoli and Ferrone 2008) and obstructs presentation of neo-antigens to cytotoxic T-cells (Garcia-Lora et al. 2003). Moreover, tumor cells may overexpress anti-apoptotic proteins such as Bcl-2 and become resistant to the cytotoxic function of immune cells (Wong 2011). The tumor microenvironment may also help in tumor cell escape by orchestrating a complex cross talk network between tumor cells and immune cells. This dynamic cross talk stimulates release of several factors by tumor and/or immune cells collaboratively which creates the immunosuppressive microenvironment. A list of factors such as transforming growth factor-β (TGF-β), vascular endothelial growth factor (VEGF), prostaglandin E2 (PGE_2), IL-10, soluble Fas, and indoleamine-2,3-dioxygenase (IDO) have been recognized as major contributors in creating the immunosuppressive microenvironment (Ben-Baruch 2006; Whiteside 2006). It has also been observed that growing tumors also recruit myeloid-derived suppressor cells and regulatory T-cells which in turn inhibit antitumor response of the host (Facciabene et al. 2012; Monu and Frey 2012; Awad et al. 2018; Togashi et al. 2019). The immune checkpoints such as PD-1 and CTLA-4 are expressed at this stage and promote immunosuppression (Teng et al. 2015). The activated T-cells express PD-1 on binding to its ligands (PDL-1, PDL-2) expressed on tumor cells which results in reduced T-cell proliferation and hence low T-cell antitumor response. Similarly, expression of CTLA-4 on the T-cell membrane and its binding to its ligands (CD80, CD86) on antigen-presenting cells inhibit co-stimulation of effector T-cells (Teng et al. 2015). Surprisingly, PD-1 and tumor-infiltrating lymphocytes are detected in colon adenoma, but PDL-1 is rarely expressed (Mostafa et al. 2016). On the other hand, other immune checkpoints such as PDL-1, PD-1, CTLA-4, IDO, and LAG-3 are expressed on tumor-infiltrating cells at the invasive front of MSI CRC (Llosa et al. 2015). In this regard, antibodies known as "immune checkpoint inhibitors (ICPIs)" have been developed to block expression of these immune checkpoints on the surface of immune cells. These ICPIs have been shown to suppress the negative signaling in T-cells triggered by molecular interactions of these ligands and their cognitive receptors in clinical studies of MSI CRC (Le et al. 2015, 2017). Taking together the above findings, it is inferred that immune surveillance plays a vital role in safeguarding the colon from CRC development from the initiation to the progression phase and represents an attractive target for prevention of this disease, i.e., CRC.

9.3 Immunomodulation and Chemosensitization of Colon Cancer

There are several strategies clinically in use for colon cancer treatment. Chemotherapy is one of them, involving use of chemotherapeutic drugs such as antimetabolites, receptor tyrosine kinase inhibitors, alkylating agents, natural products, corticosteroids, hormones, and antagonists (Pan et al. 2016). However, exposure of these drugs to tumor cells for a longer time often results in development of chemoresistance – a condition when a recommended dose of chemotherapeutic drug becomes ineffective in eradicating colon tumors (Ramos and Bentires-Alj 2015). Additionally, chemoresistance represents the main obstacle in the treatment of malignant CRC (Crea et al. 2011). Several approaches have been devised to overcome chemoresistance. Chemosensitization is one such strategy that utilizes agents of natural or synthetic origin to overcome chemoresistance and hence enhances the efficacy of conventional chemotherapeutic agents (Turrini et al. 2014). Accumulating evidences have suggested that plant-derived natural compounds are gaining substantial attention as chemosensitizers (Garg et al. 2005). These natural compounds potentiate cytotoxic capacity of drugs by modulation of multiple cellular targets with acceptable toxicity (Fig. 9.1).

In addition to chemosensitization, growing evidences have also suggested that diet has a direct impact on both adaptive and innate immune systems (Cooper and

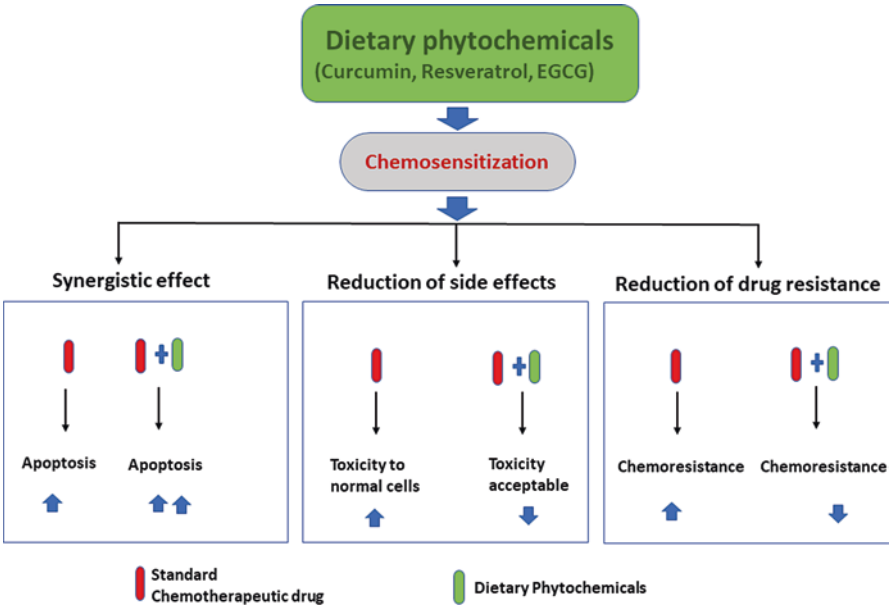

Fig. 9.1 Graphical demonstration of multiple mechanisms of chemosensitization exerted by dietary compounds to enhance the efficacy of standard chemotherapeutic drugs

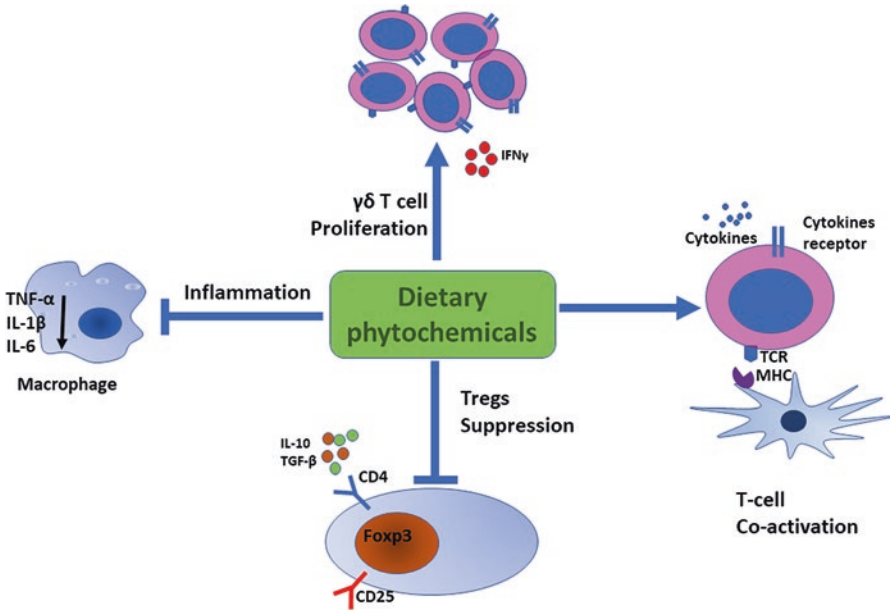

Fig. 9.2 Graphical representation of activation of multiple molecular pathways involving immune cells by dietary compounds to inhibit CRC development

Ma 2017). Several reports have indicated the antitumor effect of natural products through the modulation of immune cells including T-cells and B-cells (Soldati et al. 2018; Childs et al. 2019). The dietary bioactive compounds have been demonstrated to improve immune recognition of tumor cells, overcoming the immune escape and inhibition of inflammation (Fig. 9.2). Some of the well-known natural compounds exhibiting chemosensitizing properties are discussed as follows.

9.3.1 Curcumin

Curcumin is the principal bioactive compound found in turmeric, an Indian spice used in kitchens across the globe. The yellow color of turmeric is due to the presence of curcumin, accounting for about 2–5% of it (Tayyem et al. 2006). The use of curcumin as an anticancer, chemopreventive agent has been documented in a myriad of in vitro, preclinical, and clinical trials, owing to its selective killing of cancer cells while sparing their healthy counterparts (Lin 2007; Ravindran et al. 2009).

9.3.1.1 Curcumin as a Chemosensitizer

Growing evidences have documented the promising chemosensitizing activity of curcumin via enhancing the efficacy of several standard chemotherapeutic drugs in CRC (Bordoloi et al. 2015). Curcumin has been reported to synergistically enhance the antitumor effect of dasatinib in colon cancer via suppression of NF-κB and its downstream target genes (Nautiyal et al. 2011a). Similarly, Shakibaei et al. reported that curcumin treatment successfully reverted the 5-fluorouracil resistance of colon cancer cells (Shakibaei et al. 2013). The inhibition of the NF-κB/PI3K/Src pathway was the proposed mechanism of the chemosensitization action of curcumin in this study. Furthermore, the combination of curcumin with FOLFOX, a formulation of 5-FU and oxaliplatin, suppressed cancer stem cell-like phenotype and expression of cancer stem cell surface markers such as CD44, EGFRs, and CD166 (Patel et al. 2008; Yu et al. 2009). Curcumin-5-FU cotreatment resulted in HT-29 colon cancer cell growth inhibition via downregulating COX-2 expression (Du et al. 2005). Wang et al. reported that curcumin targets NF-κB and nuclear endonuclease G to reduce chemoresistance of platinum-derived chemotherapeutics (cisplatin, oxaliplatin, and carboplatin) in colon cancer cells (Wang et al. 2014). Moreover, Zhang et al. demonstrated that Nrf2 plays the key role in the reversal effect of curcumin on multidrug resistance in colon cancer cells (Zhang et al. 2018). The promising results obtained in in vitro studies of curcumin as a chemosensitizer encouraged its further evaluation in animal models of colon cancer. The combination therapy, i.e., curcumin + dasatinib, reduced the size of intestinal adenoma in APC $^{\min(+/-)}$ by >95% (Nautiyal et al. 2011b). Similarly, a combination of curcumin and celecoxib was given to DMH-treated mice (Lev-Ari et al. 2005). Toden et al. investigated the role of miRNA related to the epithelial-to-mesenchymal transition pathway in curcumin-mediated chemosensitization of HT-29 colon cancer cell lines to 5-FU in a mouse xenograft study (Toden et al. 2014). In this study, the chemoresistance was effectively reversed on combining curcumin with 5-FU via miRNA-induced suppression of the EMT pathway in colon cancer cells.

Taking together the results of both in vitro and preclinical studies, several clinical trials have been conducted to evaluate curcumin as a chemosensitizer in cancer patients (Bordoloi et al. 2015). Cruz-Correa et al. evaluated the potential of curcumin combined with quercetin in inhibiting the size and number of adenomas in patients suffering from familial adenomatous polyposis (Cruz-Correa et al. 2006). After 6 months of treatment, the combinatorial therapy was successful in decreasing not only the number but also the size of polyps in all the patients enrolled in the study with acceptable toxicity.

9.3.1.2 Immunomodulatory Effect of Curcumin

Curcumin exhibits anti-inflammatory activity via inhibiting key mediators of inflammation including NF-κB and COX-2 (Gupta et al. 2013). Recent reports have suggested that curcumin is capable of improving efficacy of novel treatments such

as immune checkpoint blockade. It induces apoptosis in a number of tumor systems via upregulating caspase activity in the mitochondrial pathway. It is actually known for its two well-defined immunological activities, i.e., inhibition of angiogenesis (Perry et al. 2010) and inhibition of COX-2 enzymatic levels (Nishida et al. 2006). NF-κB is a most crucial transcription factor which is responsible for the upregulation of many inflammatory genes. NF-κB exists in the cytoplasm as a heterodimer in the form of p65/p50 subunits. Curcumin nanoparticles have been shown to effectively inhibit nuclear translocation and thus activation of NF-κB both in in vitro and in vivo mouse models of colitis (Rejhová et al. 2018).

Tregs are an immune-suppressive subset of T_h cells which suppress the induction and proliferation of T_c-cells. Recent studies have emphasized the crucial role of gut microbiota in the pathophysiology of inflammatory bowel disease. In this prospect, curcumin has been shown to exert protective effects via modulating colon microbiota. Microbes such as *Clostridium cluster* IV and XIVa are an important source of butyrate, a short-chain fatty acid by-product of nondigestible carbohydrates in the colon (Sharma et al. 2018). Additionally, these bacteria have been demonstrated to induce mucosal Treg proliferation through butyrate generation (Furusawa et al. 2013). Interestingly, polyphenols have been reported to enhance the population of short-chain fatty acid-producing microbes in the colon (Espín et al. 2017). Additionally, these bacteria have been demonstrated to induce mucosal Treg proliferation through butyrate generation. A recent report suggested that administration of curcumin nanoparticles increased the efficacy of butyrate synthesis as well as inhibited DSS-induced colitis in mice (Ohno et al. 2017).

9.3.2 Resveratrol

Resveratrol is a member of chalcones family and is found as a major bioactive compound in berries, grapes, red wine, and peanuts (Gupta et al. 2011; Lissa and Castedo 2013). Epidemiological studies demonstrating the relationship of consumption of red wine and cancer incidence formulated the "French paradox" which suggested the protective effect of resveratrol – a principal bioactive compound of red wine (Renaud and Gueguen 1998).

9.3.2.1 Chemosensitizing Effect of Resveratrol

Resveratrol has been reported to chemosensitize 5-fluorouracil-resistant human CRC cell lines (HCT116, SW480) by inhibiting NF-κB and its downstream target genes (Buhrmann et al. 2015). Similarly, resveratrol potentiated the antitumor effect of etoposide on human CRC cell lines via upregulating p53 expression in a dose-dependent manner (Amiri et al. 2013). Progress in mechanisms of chemoresistance revealed NECTIN-4 as a principal mediator of 5-FU drug resistance in human CRC cell lines (Das et al. 2015). The authors demonstrated that resveratrol in

combination with BCNU (1,3-bis(2-chloroethyl)-1-nitrosourea) was able to increase 5-FU sensitivity of human CRC cell lines. Moreover, Aires et al. showed the importance of P-glycoprotein 1 expression as a crucial factor in cytotoxic activity of resveratrol in colon cancer cells (Aires et al. 2019). Recently, resveratrol in combination with ginkgetin, a bioactive compound from *Ginkgo biloba* leaf, synergistically suppressed VEGF-induced angiogenesis during colon cancer metastasis (Hu et al. 2019).

Owing to the strong anticancer and chemosensitizing activity of resveratrol, a series of clinical trials have been conducted in combination with chemotherapeutic drugs. As per https://clinicaltrials.gov to date, searching for keyword "resveratrol" showed a total of 92 clinical trials after applying two filters, *completed* and *active, not recruiting*. Most of these trials have been done to evaluate bioavailability, pharmacokinetics, biological safety, and toxicity of resveratrol. Of these, clinical trials focusing on efficacy of resveratrol alone and/or in combination with chemotherapeutic drugs in colon cancer are small in number. A summary of these trials is given in Table 9.1.

9.3.2.2 Immunomodulatory Effect of Resveratrol

With a panoramic view, resveratrol is known for its wide range of pharmacological properties including anti-inflammatory, antioxidant, as well as immunomodulatory functions. The ratio of Treg/Th17 plays an important role in the pathophysiology of colitis, and a decrease in this ratio has been reported to promote colitis in BALB/c mice (Eastaff-Leung et al. 2010). In ulcerative colitis, dose-dependent therapeutic efficacy of resveratrol has been linked to its ability to modulate the Treg/Th17 ratio and HIF-1α/mTOR signaling pathway (Mileo et al. 2019). Similarly, Yao et al. showed that resveratrol treatment restored the physiological Treg/Th17 ratio as well as suppressed the production of inflammatory cytokines and leukocyte eicosanoids as a protective mechanism against DSS-induced ulcerative colitis in mice (Yao et al. 2015). IL-10 is a cytokine of anti-inflammatory nature, and resveratrol has been reported to enhance the IL-10 production with a concomitant decrease in CD28 and CD80 without influencing the percentage of Treg cells (Švajger and Jeras 2012). Additionally, Treg cells secrete IL-10 and TGF-β to mitigate the inflammatory cascade which thus balances intestinal immune response (Unutmaz and Pulendran 2009). Hypoxia has recently been linked to the proliferation of Th17 cells and secretion of inflammatory cytokines including IL-17 (Yao et al. 2015). HIF-1α-mediated induction of Foxp3 has been reported to enhance T-cell abundance and function in the mucosa under hypoxia (Clambey et al. 2012). Treatment with HS-1793 (a resveratrol analog) has been reported to suppress the CD4+CD25+Foxp3+ population in total splenocytes and tumor tissue (Jeong et al. 2012). Moreover, resveratrol administration even at a low dose (300 ppm) exerted protective effects against DSS-induced colitis and colitis-associated colon cancer via modulation of CD3+ T cells (Cui et al. 2010).

Table 9.1 Chemosensitizing and immunomodulatory effects of dietary bioactive compounds

Dietary compound	Mechanism of action	References
Curcumin	*Chemosensitizer*	
	Promotes the antitumor effect of dasatinib in colon cancer via suppression of NF-κB and its downstream target genes	Nautiyal et al. (2011a, b)
	Combination of curcumin with FOLFOX, a formulation of 5-FU and oxaliplatin, suppresses cancer stem cell-like phenotype and also an expression of cancer stem cell surface markers such as CD44, EGFRs, and CD166	Patel et al. (2008)
	Curcumin targets NF-κB and nuclear endonuclease G for reduction of chemoresistance of platinum-derived chemotherapeutics	Wang et al. (2014)
	Nrf2 exhibits its role in the reversal effect of curcumin on multidrug resistance	Zhang et al. (2018)
	Combining curcumin with 5-FU via miRNA-induced suppression of the EMT pathway in CRC can efficiently reverse chemoresistance	Toden et al. (2014)
	Immunomodulatory	
	Enhancement of T-cell proliferation	Varalakshmi et al. (2008)
	Inhibition of FoxP3 expression and promotion of conversion of FOXP3+ Tregs into Th1 cells	Xu et al. (2017)
	Inhibition of IFN-γ secretion from CD4+ T-cells by inhibiting Treg cell function	Xu et al. (2017)
	Exhibiting anti-inflammatory activity via inhibition of NF-κB and COX-2	Gupta et al. (2013)
	Inhibition of COX-2 activity	Nishida et al. (2006)
	Inhibition of angiogenesis	Perry et al. (2010)
	Nanoparticle-conjugated curcumin directly blocks NF-κB activation in intestinal epithelial cells	Rejhová et al. (2018)
	Curcumin nanoparticles induce an expansion of Tregs in the colonic mucosa	Rejhová et al. (2018)
	Modulation of gut microbiota and mucosal Treg population	Furusawa et al. (2013)
	Curcumin exerts a biological effect in microbiota and shaping of other species	Espín et al. (2017)
Resveratrol	*Chemosensitizer*	
	Chemoresistance mechanism revealed NECTIN-4 as a principal mediator of 5-FU drug resistance in CRC cells	Das et al. (2015)
	Chemosensitizes 5-FU-resistant human colon cancer cell lines (HCT116, SW480) by suppressing NF-κB and its downstream target genes	Buhrmann et al. (2015)
	In combination with ginkgetin, synergistically suppresses VEGF-induced angiogenesis during colon cancer metastasis	Hu et al. (2019)
	Immunomodulatory	

(continued)

Table 9.1 (continued)

Dietary compound	Mechanism of action	References
	Modulated Treg/Th17 ratio and HIF-1α/mTOR signaling pathway	Mileo et al. (2019)
	Regulated the Treg/Th17 ratio to inhibit UC development	Yao et al. (2015)
	Enhanced the IL-10 production with a concomitant decrease in CD28 and CD80 expression in T-cells	Švajger and Jeras (2012)
	Suppressed CD4+CD25+Foxp3+ population in total splenocytes and tumor tissue	Jeong et al. (2012)
	Modulated CD3+ T-cell population in ameliorating DSS-induced colitis and colitis-associated colon cancer	Cui et al. (2010)
EGCG	*Chemosensitizer*	
	Synergistically enhanced the efficacy of curcumin against HCT15 and HCT116 colon cancer cells	Manikandan et al. (2012)
	Reduced the 5-FU-induced drug resistance and cancer stem cell-like characteristics of colon cancer cells	Toden et al. (2016)
	Sensitizes colon cancer cells to 5-FU via targeting MDR1, a drug efflux pump	La et al. (2019)
	Immunomodulatory	
	Inhibits the phosphorylation of signal transducer and activator of transcription 1 (STAT1) induced by IFN-γ	Ogawa et al. (2012)
	Adjust a balance between Treg and Th17 by reducing IL-6 levels	Xu et al. (2015)
	Targeting TGF-β-induced Tregs that have been shown to have unstable Foxp3 expression	Wong et al. (2011)

9.3.3 EGCG

Tea is the most widely consumed beverage across the globe. Tea is derived from plant *Camellia sinensis*, and green tea is a nonfermented product and accounts for 20% of total tea production. Epigallocatechin-3-gallate is the major polyphenol present in green tea. Epidemiological studies have suggested that regular consumption of green tea is protective against multiple inflammatory diseases including cancer (Rady et al. 2018). EGCG is the most bioactive compound present in green tea, and the anticancer potential of this compound has been documented in multiple cancers including CRC (Shukla et al. 2018).

9.3.3.1 EGCG as a Chemosensitizer

In addition to anticancer activity, chemosensitization of colon cancer cells to standard chemotherapeutic drugs with EGCG has also been investigated in various in vitro and in vivo studies (Shukla et al. 2018). Collectively, these reports indicated that green tea polyphenols especially EGCG synergize the cytotoxic activity of

many other potent anticancer phytochemicals or conventional chemotherapeutic drugs. EGCG has been reported to synergize and enhance the anticancer activity of curcumin against HCT15 and HCT116 colon cancer cells (Manikandan et al. 2012). The combination treatment led to a significant increase in DNA fragmentation, marked nuclear condensation, and apoptotic cells. EGCG has been shown to overcome 5-FU-mediated chemoresistance in colon cancer cells and cancer stem cell-like properties (Toden et al. 2016). The authors showed that EGCG led to suppression of several miRNAs playing a vital role in 5-FU drug resistance. The combination therapy was also effective in reducing the tumor size in vivo through CRC cell-based xenograft experiment. Recently, EGCG has been reported to reinforce the sensitivity of colon cancer cells to 5-FU via targeting MDR1, a drug efflux pump (La et al. 2019). The combinatorial approach of EGCG + 5-FU synergistically induced apoptosis as evidenced by increased caspase-3 activity, PARP cleavage, and reduced Bcl-2 expression. Overall, the study suggested that EGCG chemosensitizes the colon cancer cells to 5-FU via the GRP78/NF-κB/miR-155-5p/MDR1 pathway.

Based on encouraging results of in vitro studies regarding EGCG as a chemosensitizer, in vivo studies were performed to translate the efficacy of EGCG as a chemosensitizer. The in vivo studies of EGCG in combination with sulindac, a preventive medicine for colon cancer, not only reduced the tumor number approximately by 50% but also reduced the size of tumors in min mice (Suganuma et al. 2001). Similarly, 0.01% EGCG in drinking water and sulindac treatment 10 mg/kg body weight (thrice a week) also showed a synergistic effect in reducing azoxymethane-induced ACF formation in the mouse colon (Ohishi et al. 2002).

Several clinical trials were conducted to evaluate the usefulness of tea polyphenols in preventing cancer. Green tea extract enriched in catechins has been demonstrated to prevent or suppress the tumor progression in patients suffering from cancers of multiple origin (Chikara et al. 2018).

9.3.3.2 Immunomodulatory Effect of EGCG

Colorectal cancer cells show a property of "immune escape" via upregulating indoleamine-2,3-dioxygenase (IDO), the tryptophan catalytic enzyme which offers a great advantage in progression of tumor and evading immune responses. IDO may also inhibit T-cell and natural killer cell proliferation. EGCG inhibits IDO protein expression by activation of IFN-γ (Ogawa et al. 2012). EGCG also inhibits the phosphorylation of signal transducer and activator of transcription 1 (STAT1) induced by IFN-γ. Overall, it is considered as the most potent natural compound which effectively serves as an antitumor immunotherapy.

Tregs are important for regulating autoreactive cells. In the context of cancers, Treg cells are a $CD4^+$ T-cell subset which are highly suppressive in nature and help cancers for their progression. Self-reactive immune cells in autoimmunity are detrimental, and dampening their response is a preferential task. That's why self-reactive cells in cancers lead to antitumor activity. Keeping this point of view, EGCG may

be considered as a valuable compound for modulating self-reactive cells for targeting "off side effects" which occurred in cancer chemotherapeutics. EGCG affects the differentiation of naive $CD4^+$ T-cells into different effector subsets. Further, EGCG was shown to adjust a balance between Treg and Th17 by reducing IL-6 levels and thus appears to be a promising treatment for ulcerative colitis (Xu et al. 2015). However, in vitro studies suggested that TGF-β-induced Tregs have been shown to have unstable Foxp3 expression (Wong et al. 2011). The collective information on immunomodulatory and chemosensitizing capacity of abovementioned dietary phytochemicals has been given in Table 9.1.

9.4 Conclusion

This chapter has discussed the beneficial role of dietary phytochemicals and their bright future as anti-inflammatory, chemopreventive, immunomodulatory, and chemosensitizing agents in CRC treatment. The regular consumption of bioactive dietary compounds not only prevents CRC development but also stimulates the immune system to exercise effective immune surveillance. Recent evidences have also suggested the use of dietary agents as chemosensitizing agents for chemotherapeutic drugs or radiotherapy opening the window of a new combination therapy capable to reduce adverse side effects and toxicity of conventional treatments for CRC. Further studies are required to evaluate the efficacy of dietary compounds alone and/or in combination with novel CRC treatment such as immune checkpoint blockade.

9.5 Future Directions

The growing evidences of gut microbiota playing a pivotal role in the pathogenesis of CRC have allured the scientific community in recent times. On the other hand, several epidemiological and experimental studies have demonstrated the potential of dietary phytochemicals in preventing CRC development. Symbionts and commensal bacteria in the gut interact with the host immune system which plays an important role in immune function. Therefore, it would be interesting to understand the role of various dietary phytochemicals in maintaining a specific population of symbiotic bacteria in the gut, whose metabolic products boost the immune system. Additionally, identification of particular microbiota enriched in the gut on consumption of a specific dietary agent would be a promising approach in understanding the mechanistic axis involving dietary phytochemicals, immune system, gut microbiota, and CRC prevention.

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Chapter 10
Role of DNA Mismatch Repair Genes in Colorectal Cancer

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Abstract Colorectal cancer (CRC) is the third most known type of cancer in developing countries and is one of the leading causes of deaths around the world. The current treatment therapies for CRC are increasing nowadays since there are large variations seen among individuals in terms of diagnosis and response to drugs used to manage CRC owing to biomolecular heterogeneity. Around 15% of CRC cases are due to defect in the DNA mismatch repair (MMR) system, featured by microsatellite instability (MSI) characteristics. We highliht the role of DNA mismatch repair genes in CRC, its diagnosis and management including targeted therapies and immune checkpoint blockade therapies for controlling the individual CRC therapy response and treatment.

Keywords Colorectal cancer · Mismatch repair · Microsatellite instability · Targeted therapy

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Abbreviations

5-FU	5-Fluorouracil
CRC	Colorectal cancer
HNPCC	Hereditary non-polyposis colorectal cancer
MLH1	MutL homolog 1
MLH3	MutL homolog 3
MMR	Mismatch repair
MSH2	MutS homolog 2
MSH6	MutS homolog 6
MSI	Microsatellite instability
MSI-high	Microsatellite instability-high
MSI-low	Microsatellite instability-low
MSS	Microsatellite stable
PCNA	Proliferating cell nuclear antigen
PD-L1	Programmed death ligand-1 protein
PMS1	PMS homolog 1
PMS2	PMS homolog 2
RFC	Replication factor C
RPA	Replication protein A

10.1 Introduction

In various aspects, the genetic and molecular basis for tumour establishment and progression is not clearly understood. The fact that cancer is a polygenic disorder and is caused by diverse mutational factors governing specific genes is now well established. Subsequently, cancer nowadays is not apparently a singular abnormal condition but constitutes an assortment of diseases controlling certain genetic heterogenic profiles. The key players controlling cellular processes such as cell proliferation, differentiation, regulation, apoptosis and immune responses could be specifically targeted in cancers, envisioning certain mutational profiles in cells (Somarelli et al. 2020). Moreover, deformity in any of the cellular signalling mechanism would lead to tumour progression and evolution. The two major categories for cancer genes available are oncogenes and tumour suppressor genes. Oncogenes are a group of genes controlling significant signalling mechanisms and are usually altered through genetic modifications, activating protein counterparts and finally leading to increased cellular proliferation. On the other hand, tumour suppressor genes harbour characteristic genome alterations such as deletions, insertions and loss-of-function mutations, thus inactivating the biological functions. However, most of the tumour suppressor genes are involved in DNA repair mechanisms occurring during cell cycle divisions. Any modifications in the DNA repair genes, however, would not stimulate the cellular proliferation process but rather increase

tumourigenesis in cells, thus promoting cancer development. However, our understanding of cancer-related genes is exponentially increasing to better understand the molecular mechanism for improving the prognosis and diagnostic treatment strategies as an ultimate goal to advance the patient requirements in combating cancer. Furthermore, while considering major cellular mechanisms involved in cancer disease, one should not forget about DNA repair mechanisms associated with cancer.

In the present chapter, we will be focussing on the mismatch repair (MMR) mechanism which is majorly involved in maintaining genomic stability and discuss its role in cancers, especially colorectal cancer (CRC). When MMR fails to function normally, modifications in microsatellite repeats occur, thereby increasing the mutational frequency of a cell as a whole. Hence, the MMR mechanism plays a vital role in cancer development and aetiology. The MMR-related associated genes and their role in microsatellite instability in cancer have various clinical and therapeutic inferences which could be targeted in future immunological studies for better prognosis.

10.2 Mismatch Repair (MMR) Mechanism

Various reviews are available on the human DNA mismatch repair (MMR) mechanism (Buermeyer et al. 1999; Jiricny 2006). The MMR process is a post-replication mechanism that is involved in conserving the DNA homeostasis and stabilizing the genome complexity (Kunkel 2009). The preliminary role of this process is to repair and eradicate the single base-pair mismatches as well as insertions and deletions which might be arising due to escape from proofreading during the DNA replication process. In addition, the MMR mechanism is required for correcting small insertion or deletion loops which might occur during the replication process while moving through repetitive sequences, called microsatellites. If the cell escapes the proper DNA repair mechanism, this erroneous mutation would lead to modification in cellular behaviour and might promote tumourigenesis. In addition, abnormality in the MMR system due to loss of function of any of its chief players is connected with increased tumour progression as well as microsatellite instability.

In general, the DNA repair mechanism gets disturbed when the MMR proteins are malformed or deficient and fails to correct the spontaneous mutations such as nucleotide mispairing and insertion-deletion loops, inserted during DNA replication. The increased mutational events in a cell arise due to alterations occurring in sequences within the microsatellite regions; however, variations in these repetitive sites cause microsatellite instability which is a major characteristic of tumour cells (Schmidt and Pearson 2016). Besides microsatellite instability, another type of instability occurring in tumour cells augmenting tumourigenesis is known as chromosomal instability which is connected with chromosomal and structural deformed arrangement during the mitotic anaphase of the cell cycle (Bach et al. 2019). Chromosomal defects are a characteristic feature of cancer disorders, finally leading to aneuploidy defects in the upcoming generations of cells (Sansregret and Swanton

2017). In addition, modifications in oncogenes such as *myc* and *ras* and tumour suppressor genes such as *APC* are also causal factors for chromosomal instability, mainly due to the occurrence of somatic mutations. DNA synthesis during replication involving DNA polymerase enzymes is generally an error-prone process with an error frequency estimated around 1 error per 10^5 nucleotide bases incorporated, leading to ~1 million errors integrated during each S phase of the cell cycle (Bębenek and Ziuzia-Graczyk 2018; Kunkel 2009). The primary defence against such a great mutational frequency is the proofreading mechanism embraced by DNA polymerase enzymes. However, some unseen proofreading activity could be missed by any of the DNA polymerase domains which needs to be corrected through a secondary defence mechanism known as MMR-associated genes.

In order to mediate the DNA repair mechanism, various adaptable proteins jointly called as MSH and MLH/PMS proteins, functioning as heterodimers, have been effectively evolved in eukaryotic species including humans. The MMR pathway in brief involves four major processes: recognition of mismatched bases or insertion-deletion loops in DNA, deletion of these lesions, incorporation of correct bases in place of these errors and finally ligation of DNA fragments (Huang and Li 2018). The MMR process is employed by a majority of bacteria and in humans, it is mainly controlled by a group of six proteins comprising MutL homolog 1 (MLH1), MutS homolog 2 (MSH2), MutL homolog 3 (MLH3), MutS homolog 6 (MSH6), PMS homolog 1 (PMS1) and PMS homolog 2 (PMS2). The mismatch recognition is performed by the MSH2 protein forming a complex with the MSH6 or MSH3 protein of the MMR pathway based on whether single base-pair mismatches or large insertions or deletions, are to be corrected (Das Gupta and Kolodner 2000; Marsischky et al. 1996). After MSH2 binds to a DNA lesion, initiation of the binding of MutL occurs which comprises heterodimer formation between MLH1 and PMS2 proteins, thereby causing recruitment of other additional proteins including DNA polymerases δ and ε, single-stranded DNA-binding proteins, replication protein A (RPA), proliferating cell nuclear antigen (PCNA), replication factor C (RFC), exonuclease 1, FEN1 (RAD27), and DNA polymerase-associated exonuclease enzymes and finally DNA ligase for religation of DNA fragments, thus completing the DNA repair process (Fig. 10.1). Once the DNA lesion is identified; and MutS and MutL heterodimers are attached to it, the DNA repair process is initiated with activated DNA exonuclease enzymes mediating further degradation of the DNA fragment from the lesion situated around 1–2 kb from the mismatch. Degradation is continued until the mismatch part is repaired and excised. The resultant large removed part is filled in by DNA polymerase enzyme δ which then incorporates a correct nucleotide in the sequence. The MMR proteins, in addition, are also known to be involved in processes such as DNA recombination, cell cycle controls at G1 and G2-checkpoints, apoptosis and cell cycle arrest in response to any certain kind of DNA modification (Jiricny 2006).

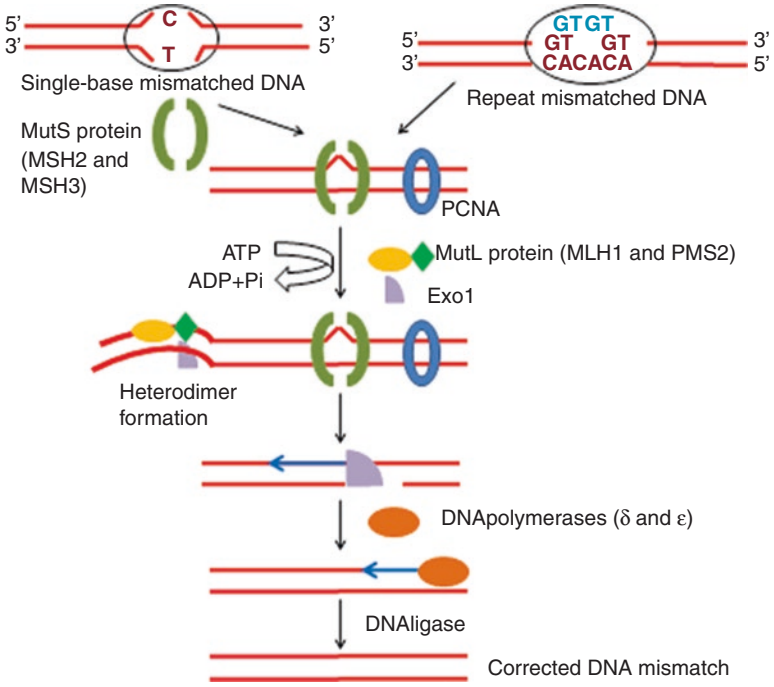

Fig. 10.1 Schematic representation of the mismatch repair pathway

10.3 Role of MMR Deficiency in Developing CRC

The present findings suggest that an abnormal MMR system is present in around 15–20% of total CRC cases reported (Petrelli et al. 2019) which could be detected by the presence of microsatellite instability (MSI) (Fig. 10.2). They are small sequence repeats of around two to five base-pair motifs, distributed throughout the genome. The MSI signifies areas error-prone due to mismatches incorporated during the DNA replication process, and thus a deficient MMR system could be recognized by screening fallacies in microsatellite development. Dysfunctionality in repair proteins such as MLH1, MSH2, MSH6 and PMS2 leads to MSI and could be inherited as germline variations in the case of the Lynch syndrome or could be sporadic when it occurs due to MLH1 promoter hypermethylation or biallelic mutation in the repair genes (Haraldsdottir et al. 2014). Such MMR defects might occur due to diverse factors including certain mutation inherited in MMR-related genes, somatic alteration in MMR genes, expression or suppression of MMR genes due to epigenetic modifications or a mixture of these features (Imai and Yamamoto 2008). The MMR genes are usually known to function as tumour suppressor genes, where loss of function of both the allelic sites is needed for failure of the tumour suppression effect. The deficient MMR system can be examined either by failure of immune histochemical staining of repair proteins or by comparing PCR-detected mutations

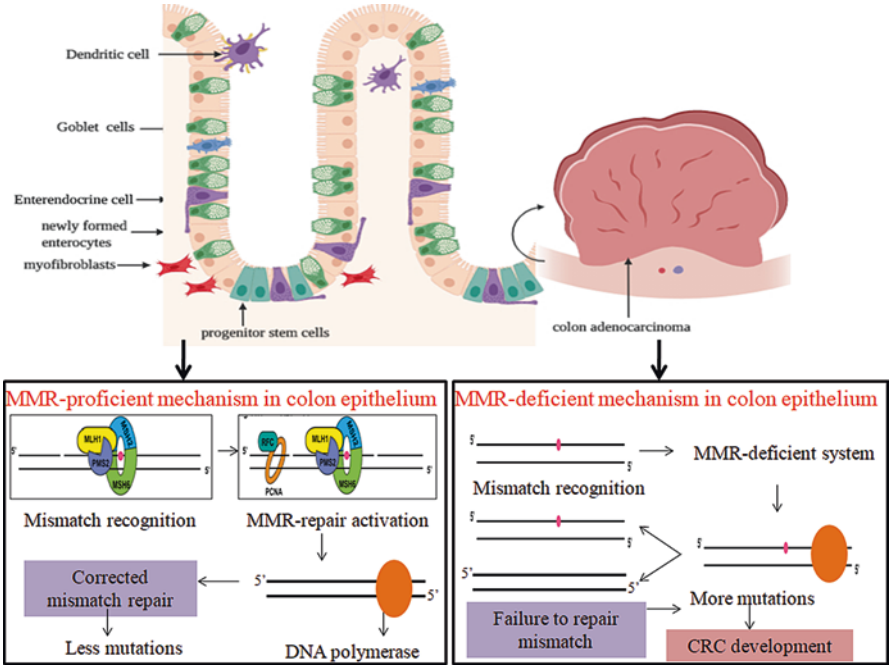

Fig. 10.2 MMR-proficient and MMR-deficient mechanisms in colon cells leading to colorectal cancer

in microsatellites between CRC tumour patients and healthy individuals (Umar et al. 2004).

Genetic susceptibility to several autosomal recessive and dominant disorders including colorectal cancer (CRC) syndromes has been well characterized and mapped for causal mutations (Farrington et al. 2005). Majority of autosomal dominant disorders are characterized on the basis of familial inheritance patterns and clinical measures which have been considered for hereditary non-polyposis colorectal cancer (HNPCC), also known as the Lynch syndrome (Umar et al. 2004). However, applying such approaches creates bias towards reduced penetrant variants, and novel mutations. The Lynch syndrome mostly occurs due to inactivating mechanisms in DNA mismatch repair (MMR) genetic mutations, especially in MLH1, MSH6 and MSH2 (De La Chapelle 2004) genes, but CRC patients carrying such mutations do not fulfil the criteria for having the Lynch syndrome as well (Hampel et al. 2005). The poorly developed sporadic MMR system is represented in the majority of CRC cases (15% of total CRC cases), and the chief reason is driven towards rapid suppression of MLH1 transcriptional activity which further leads to hypermethylation of its promoter sites (Evrard et al. 2019) (Fig. 10.3). In addition, an approximate 1 in 3000 individuals (aged between 15 and 75 years) harbour abnormal DNA MMR genes (Dunlop et al. 2000) suggesting that individuals having CRC and fulfilling the criteria for the Lynch syndrome do not necessarily report for

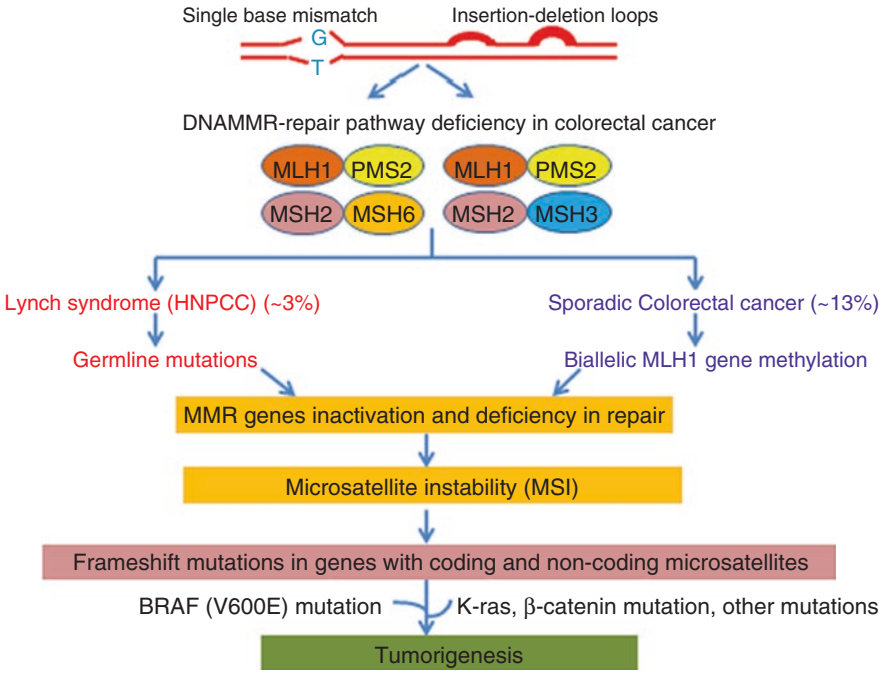

Fig. 10.3 Illustration for molecular pathways involved in colorectal cancer and DNA mismatch repair with microsatellite instability

the entire mutational carrier genes. Even the non-symptomatic mutational carriers have a significant risk of CRC which accentuates the value of determining such carriers at an early stage to allow for better prognosis and treatments (Quehenberger et al. 2005).

However, there are unusual characteristic clinical features associated with a defective MMR phenotype, such as 40% of patients with the Lynch syndrome who cannot be diagnosed only on the basis of morphological characters (Alexander et al. 2001). Deliberate arguments exist regarding the diversity in morphological features between CRC and the Lynch syndrome in association with the defective MMR system (Gatalica and Torlakovic 2008; Jass 2004). Moreover, few groups have given inferences about the close linkage of Lynch syndrome tumours with medullary and mucin characters, while others have suggested the presence of mucinous characters along with deprived cellular differentiation in sporadic abnormal MMR tumours (Gatalica and Torlakovic 2008). On the other hand, CRC is usually seen in serrated polyps and is associated with variation in oncogene *BRAF* (V600E), older age and gender of the individual. These evidences suggest that the presence of diversity in phenotypic characters might be expected between Lynch syndrome tumours and sporadic deficient MMR CRC tumours (Ogino and Goel 2008).

10.4 Approaches for Identifying Deficient MMR

Diverse approaches are available for identifying deficient MMR proteins in tumour cells. The variations in MMR genes could be detected through DNA sequencing methods, while the presence of MMR genes could be determined through immuno-histochemical analysis of tumour tissues in colon-related disorders (Palomaki et al. 2009; Shia 2008). Moreover, the mutational phenotype in CRC patients could be examined by assessing the MSI prominence in tumour cells. Due to the presence of repetitive sequences in microsatellite regions, increased susceptibility to replication errors occurs in these regions caused by failure of proper functioning of DNA polymerases across repeats. Such replication errors could be repaired by the DNA MMR mechanism, but in the defective MMR pathway, however, the errors become constant and the size of microsatellite regions gets modified. The MSI could be easily examined in excised tumour tissues from patients which has been also accepted worldwide according to international guidelines (Umar et al. 2004; Zhang 2008; Phelip et al. 2019). The tumours are classified as microsatellite instability-high (MSI-high), microsatellite instability-low (MSI-low) and microsatellite stable (MSS) on the basis of stability of microsatellite markers present in a panel. MSI-high is where more than two instable microsatellite markers are present in a panel of five markers, while MSI-low is where one of the total five markers is positive for instability and MMS represents a condition for microsatellite stability. However, some have given a classification for MSI-low as a unique pathological phenotype, while others have included it as MSS (Ogino and Goel 2008). MSI has been found to correlate with loss-of-function mutations in MLH1, MSH2 and PMS2 genes, while evidences reported for mutation in the MSH6 gene do not necessarily exhibit MSI occurrence, most likely due to functional lay-off of the hMutSα gene. Thus, extra studies are required to analyse tumour cells, if MSI is not observed but MSH6 defect is alleged from familial inheritance cases (Yang et al. 2019). Few guidelines have been updated and developed for estimating patients at risk of the Lynch syndrome and CRC due to presence of MSI and deficient MMR systems. Additionally, there have been attempts made to predict the mutational spectrum of the *BRAF* oncogene for V600E variation and methylation prominence of the MLH1 gene which could be used further to rule out the individuals suffering from sporadic defective MMR (Adelson et al. 2011; Harada and Morlote 2020; Julié et al. 2008).

10.5 Management of the Deficient MMR System in CRC

The deficient MMR system represents a distinct type of cancer aetiology and pathogenesis which may also depict response to therapeutic applications. The suggested management for patients with the Lynch syndrome involves rapid colonoscopic

screening (1–2 years) than standardized care followed by preliminary CRC treatment (3 years). However, collective colectomy has been promoted in comparison to regular colonoscopy treatment as it is connected with poor quality of life with rectum examination required as well (Van Duijvendijk et al. 2000; Lynch et al. 2008). In the present scenario, neoantigen adjuvant therapies are utilized for CRC management (Chau et al. 2006) which could further lead to proper management in terms of providing a space for test diagnostic material so that clinical management could be properly informed.

A few studies have reported differing outcomes stating defective MMR associated with MSI as an efficient prognostic marker linked with favourable results in CRC patients (Benatti et al. 2005; Popat et al. 2005; Zhao et al. 2019). These studies have reported that the MSI-associated CRC was linked with a considerably enhanced existence rate in patients regardless of CRC stage. In addition, patients using 5-fluorouracil agent had showed improved prognostic treatment in existence of MSI (Carethers et al. 2004; Popat et al. 2005). Furthermore, multiple evidences regarding favourable outcomes in defective MMR tumours have been observed in other large cohort studies (Braun et al. 2008; Koopman et al. 2009). Although only 4% of metastatic cancers exhibit MSI in comparison to 15–18% of major cancers, this emphasizes the fact that a defective MMR system is linked with a less intimidating record.

However, there remain some limitations to this surveillance. First, CRC associated with mutation in oncogene *BRAF* (V600E) has been reported with poor surveillance outcomes in few studies (Lochhead et al. 2013; Ogino et al. 2009). Particularly, connection between the *BRAF* (V600E) gene and sporadic defective MMR system was observed to have lower prognosis in such cases than familial cases (Loughrey et al. 2007). This has been reported in a case study where patients with MSI and *BRAF* (V600E) mutation were analysed and tumours were treated with an adjuvant chemotherapy approach. In few studies, patients with MSI and without *BRAF* (V600E) mutation had shown significantly improved prognosis, while tumour patients exhibiting both MSI and *BRAF* (V600E) mutation had shown similar survival rate to those without encompassing MSI tumours (French et al. 2008; Taieb et al. 2017). A second limitation is concerned about chromosomal instability in CRC tumours. It is well understood that microsatellite stability is related with chromosomal structural deformities and aneuploidies. However, MSI and chromosomal instability are not commonly associated (Trautmann et al. 2006); they are inversely linked with each other. In addition, chromosomal instability is a reduced prognostic marker for CRC tumours which is subsequently reported by a study where the prognostic effect of MSI gets diminished when chromosomal instability and other chromosomal deformities are involved (Sinicrope et al. 2006; Walther et al. 2008).

10.6 Defective Mismatch Repair in Response to Targeted Therapies

Spontaneously, the efficiency of the DNA MMR system could be related with direct impact on cellular mechanisms mediated through DNA damage, as caused by chemotherapeutic substances, and certainly might alter the damage detection by the apoptotic system. On the other hand, it is also possible that mutation accumulation in the cells in consequence of a defective DNA MMR system might lead to activation of cytotoxic agents. Undoubtedly, the MMR system has been involved in repairing the DNA damage and subsequently arresting the cell cycle and activation of apoptosis mechanism in response to damage (Bignami et al. 2003). However, current medical treatment strategies include a mixture of various chemotherapeutic agents including fluoropyrimidines, platinum compounds, methylating agents and topoisomerase inhibitors which have been elucidated in several studies (Cremolini et al. 2015; Bruno et al. 2017; Ray et al. 2019). These genotoxic drug agents are known to directly or indirectly stimulate DNA damage mechanism which is predicted by a specific DNA repair system. For instance, oxaliplatin is a specific platinum compound which induces cell apoptosis via several mechanisms including activation of the ribosome stress-induced biological process (Bruno et al. 2017). Another drug metabolite named as 5-fluorouracil (5-FU) inhibits the thymidine synthase enzyme which is involved in the DNA synthesis process and is alleged to hinder the DNA replication process, thus causing DNA mismatches which are identified and repaired by the DNA MMR system (Iwaizumi et al. 2011). Another nucleotide analogous compound known as TAS-102 obstructs DNA synthesis in a similar manner to the 5-FU compound and is efficient for those CRC patients who are resistant to targeted 5-FU therapy (Lenz et al. 2015). In particular, these compounds are reported effective in cells with defective repair pathways, and CRC patients carrying mutations in these repair genes could be identified as definite response to these kinds of targeted therapies (Fig. 10.4).

The CRC tumours are known to have excessive neoantigen development due to a deficient MMR system besides greater programmed death ligand-1 protein (PD-L1) expression (Germano et al. 2018; Kim et al. 2016). However, recent reports have suggested that MSI tumour cells have shown positive response towards immune checkpoint blockade systems including PD-1 and CTLA-4 (Le et al. 2017; Luksza et al. 2017). In a study reported by Wang et al., CRC patients with increased expression of tumour-infiltrating lymphocytes and the PD-L1 protein have responded well to the checkpoint blockade system in comparison to patients with reduced levels of PD-L1 expression, suggesting that it could be utilized as an interpreter of treatment response in such patients (Wang et al. 2018). In phase II clinical trials, the nivolumab drug which is a PD-L1 immune system checkpoint blocker has shown longer survival response in MMR-deficient and MSI-high CRC patients (Overman et al. 2018). Furthermore, this drug was FDA approved and was considered suitable for treatment of CRC patients with defective MMR systems and MSI (Sarshekeh et al. 2018). Moreover, the CRC patients with defective MMR systems

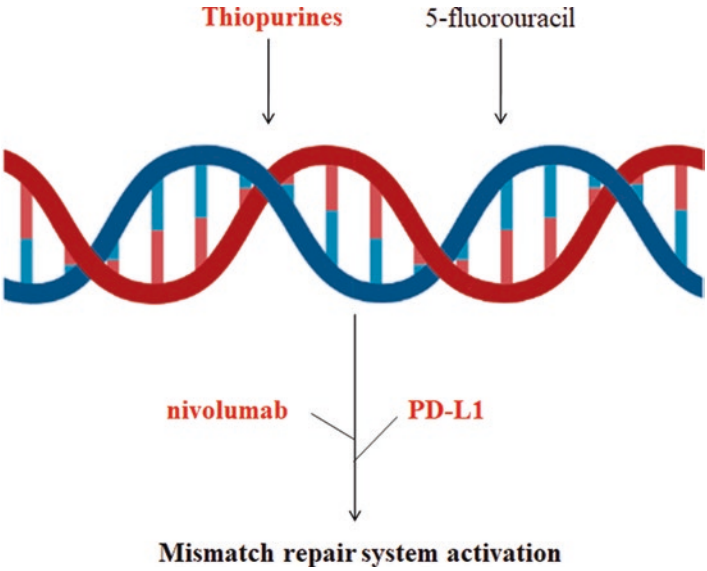

Fig. 10.4 Targeted chemo- and immune therapy for DNA mismatch repair activation in colorectal cancer patients

have elevated levels of mutational burden which could also be found in a small cohort of tumours with MSS. In an analysis report of over 6000 CRC patients, around 95% of tumour cases were found to be MSS but with the increased mutation burden phenotype; and in this group of tumours, around 55% responded better to anti-PD-L1 immunotherapy treatment (Fabrizio et al. 2018). These outcomes suggest that in patients with defective MMR systems with MSS, there is an increased mutational burden which could be considered as an efficient marker for treatment response in tumour checkpoint blockade therapy.

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Chapter 11
Targeting of Aerobic Glycolysis: An Emerging Therapeutic Approach Against Colon Cancer

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Abstract Colon cancer is one of the leading causes of cancer-associated deaths in men as well as in women worldwide. Therefore, various researches are being conducted to identify suitable therapeutic targets for designing the safer and effective therapeutic regimens against colon cancer. In view of this, aerobic glycolysis has been identified as one of the prominent and potential therapeutic targets for the treatment of colon cancer. Interestingly, overwhelming reports suggest that not the oxidative phosphorylation (OXPHOS) but rather glycolysis is one of the major sources of energy production in colon cancer even in the presence of sufficient oxygen. Hence, the "Warburg effect" or "aerobic glycolysis" is among the most detectable features in colon cancer which directly or indirectly mediates other hallmark features. This metabolic switch benefits colon cancer in several ways with respect to its development and progression, which include promotion of macromolecular synthesis, evasion of apoptosis, drug resistance, and immunosuppression. In colon cancer, mutations in Wnt, p53, and Ras play a critical role in switching the glucose metabolism from mitochondrial oxidative phosphorylation to cytoplasmic glycolysis. Overall, targeting of aerobic glycolysis by synthetic or natural compounds may help in designing the novel therapeutic approaches for the treatment of colon cancer.

Keywords Altered glucose metabolism · Glycolytic inhibitors · Metabolic switch · Phytochemicals

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Abbreviation

3-BrPA	3-Bromopyruvate
ABCB1	ATP-binding cassette, subfamily B, member 1
AMPK	5′ adenosine monophosphate-activated protein kinase
APC	Adenomatous polyposis coli
AT-1	Atractylenolide 1
BRAF	B-Raf
CRC	Colorectal cancer
CSN	COP9 signalosome complex subunit 5
DCA	Dichloroacetate
GIST	Gastrointestinal stromal tumors
GLUTs	Glucose transporters
KRAS	Kirsten rat sarcoma viral oncogene homolog
LDHA	Lactate dehydrogenase A
LPA	Lysophosphatidic acid
MIF	Macrophage migration inhibitory factor
NADPH	Nicotinamide adenine dinucleotide phosphate
PDH	Pyruvate dehydrogenase
PDK	Pyruvate dehydrogenase kinase
PDP	Pyruvate dehydrogenase phosphatase
PFK	Phosphofructokinase
PHLPP	pH domain leucine-rich repeat protein phosphatase
PK	Pyruvate kinase
PPP	Pentose phosphate pathway
SGLT	Sodium-dependent glucose cotransporters
TGF-β	Tumor growth factor-β
TME	Tumor microenvironment
TRPC5	Transient receptor potential canonical channel 5
VEGF-A	Vascular endothelial growth factor A
α-CHC	α-Cyano-4-hydroxycinnamate

11.1 Introduction

Colon cancer is a type of gastrointestinal cancer which occurs in the colon. The colon, also known as the large bowel, is a long, coiled tubelike organ whose one end is associated with the small intestine and the other with the anus. The colon and rectum along with the anus collectively form the large intestine, the last segment of the gastrointestinal tract. The large intestine serves as one of the important parts of the digestive system and helps in the partial absorption of water and minerals from the digested food. It also harbors niche for several microorganisms. Generally, colon and rectal cancers have similar clinical features; hence, they are collectively

called as "colorectal cancer." It primarily originates from epithelial or glandular cells of the large intestine. Worldwide, colorectal cancer is reported as the fourth most frequently diagnosed cancer (Siegel et al. 2020). Moreover, it is globally recognized as the third leading death-causing cancer (Siegel et al. 2020). In 2018, according to the World Health Organization, 1.8 million new cases of colorectal cancer were confirmed along with approximately 861,000 deaths (Bray et al. 2018).

Colorectal cancer (CRC) can be caused by several factors such as family history of colon and rectal cancers in parents and siblings, history of high risk of adenomas (colorectal polyps with transformed cells), inherited mutated genes that increase the risk of familial adenomatous polyposis (FAP) or Lynch syndrome (hereditary non-polyposis colorectal cancer), history of chronic ulcerative colitis or Crohn disease for 8 years or more, and huge alcohol consumption (Freeman 2008). Gastrointestinal stromal tumors (GIST) also contribute to the occurrence of colon cancer (Reddy and Fleshman 2006). Other factors which may contribute to the development and progression of CRC are type of diet intake and digestive tract inflammatory situations (Ryan-Harshman and Aldoori 2007; Thanikachalam and Khan 2019; Boland et al. 2005; Rubin et al. 2012).

Despite the advancements in diagnosis and therapeutic approaches, in recent years, neither the incidence nor the death rate of CRC has declined. Therefore, researchers are trying to identify novel therapeutic targets for the development of effective and efficient therapeutic approaches for the treatment of CRC. Interestingly, several recent studies have shown altered glucose metabolism in CRC (Brown et al. 2018; Fang and Fang 2016). Reports suggest that colorectal cancer cells display a great dependency on the glycolytic pathway for the fulfillment of their energy requirement even in the presence of ample oxygen, probably due to mutations in genes like p53, Wnt, and Ras, which are widely reported in inducing the metabolic switch in glucose metabolism from oxidative phosphorylation to glycolysis in other cancers also. Therefore, targeting of altered glucose metabolism could be an effective therapeutic strategy for the treatment of CRC.

In this book chapter, we have illustrated the role of aerobic glycolysis in the development and progression of colon/colorectal cancer. Further, we have discussed the importance of targeting aerobic glycolysis either by natural or synthetic compounds for the development of better and effective therapeutic regimens for the treatment of colon/colorectal cancer.

11.2 Role of Aerobic Glycolysis in Colon Cancer Progression

11.2.1 Glycolysis as an Instant Source of Energy and Macromolecular Precursors

Glycolysis is the most common pathway for energy production in prokaryotic as well as eukaryotic cells. Earlier, glycolysis was known as one of the critical intermediate pathways of energy production through glucose metabolism either in the

presence or absence of oxygen. However, vigorous studies and advancement in technology have shown that it also participates in the regulation of amino acid, lipid, and nucleotide biosynthesis through providing its intermediate by-products as precursor molecules. Glycolysis is tightly regulated by several enzymes, including hexokinase II (HKII), phosphofructokinase (PFK), pyruvate kinase (PK), and pyruvate dehydrogenase kinase (PDK). Interestingly, neoplastic cells usually exploit the glycolysis pathway for the generation of ATPs even in the presence of abundant oxygen. This phenomenon is popularly known as "aerobic glycolysis" and was first time observed by Otto Warburg and thus also called as the "Warburg effect" (Warburg 1956). The cancerous cell switches glucose metabolism from oxidative phosphorylation (OXPHOS) to glycolysis, as this metabolic switch provides several benefits to the cancerous cell such as evasion of mitochondria-dependent apoptosis, invasion and metastasis, angiogenesis, immunosuppression, and chemoresistance (Gatenby and Gillies 2004; Liberti and Locasale 2016; Lin et al. 2019). Further, cytoplasmic glycolysis only provides a small number of ATPs (two ATPs) in contrast to mitochondrial oxidative phosphorylation (30–32 ATPs per glucose molecule). To overcome this problem, the cancer cell dramatically increases the rate of ATP production with glycolysis by 100 times compared to mitochondrial oxidative phosphorylation (Ganapathy-Kanniappan and Geschwind 2013). Colorectal cancer cells display highly accelerated glycolysis due to overexpression/activation of key glycolytic regulatory molecules, namely, glucose transporters 1 and 3 (GLUT1 and GLUT3), hexokinase II (HKII), hypoxia-inducible factor-1α (HIF-1α), pyruvate kinase muscle isozyme M2 (PKM2), monocarboxylate transporter 4 (MCT4), lactate dehydrogenase A (LDHA), and pyruvate dehydrogenase kinases 3 and 4 (PDK3, PDK4) (Wang et al. 2015a; Gregersen et al. 2012; Brown et al. 2018; Fang and Fang 2016). Further, elevated glycolysis also enhances the rate of the pentose phosphate pathway (PPP) via supplying glucose-6-phosphate, the product of the first enzymatic reaction of the glycolysis pathway (Liberti and Locasale 2016). The pentose phosphate pathway plays a very essential role in the anabolism of lipid and nucleic acid via synthesizing NADPH and ribose-5-phosphate. In addition, other by-products of glycolysis which include glyceraldehyde-3-phosphate and 3-phosphoglycerate provide preparatory molecules for lipid and amino acid biosynthesis pathways (Liberti and Locasale 2016). Taken together, the above-mentioned studies suggest that abruptly enhanced glycolysis supports growth and development of CRC through immediately satisfying the energy demand and supplying precursor molecules for amino acid, lipid, and nucleotide biosynthesis pathways.

11.2.2 Signaling Pathways Responsible for Reprogrammed Glucose Metabolism in Colon Cancer

The elevated expression of glycolysis regulatory molecules in CRC is possibly the consequence of mutations in tumor suppressor genes and proto-oncogenes resulting in altered signaling pathways such as Wnt, RTK-RAS, and p53 signaling pathways (Brown et al. 2018; La Vecchia and Sebastian 2020).

Reports suggest that most of the sporadic colorectal tumors display a hyperactivated Wnt signaling pathway due to inactivation of the tumor suppressor adenomatous polyposis coli (APC) gene and activation of β-catenin (Schatoff et al. 2017; Sebio et al. 2014). Interestingly, studies have shown that the hyperactivated Wnt signaling pathway either directly or indirectly through MYC is involved in the upregulated expression of several glycolysis regulatory molecules, including PDK1, MCT1, GLUT1, and PFK1 (Brown et al. 2018; Osthus et al. 2000; Satoh et al. 2017). Thus, the hyperactivated Wnt signaling pathway may have a role in acquiring the Warburg metabolic phenotype provided to colorectal cancer cells.

The RAS-RTK signaling pathway is one of the important proliferative signaling pathways, which is found altered in many cancers, including CRC (Brown et al. 2018; Saeed et al. 2019). Reports indicate the pivotal role of mutated GTPase KRAS and its downstream target BRAF (a serine-threonine protein kinase) in the pathogenesis of CRC (Larki et al. 2017; Midthun et al. 2019). Intriguingly, recent studies suggest the role of oncogenic KRAS and BRAF mutations in providing the Warburg metabolic phenotype to the colorectal cancer cells (Fang and Fang 2016; Hutton et al. 2016; Brown et al. 2018). The oncogenic KRAS and BRAF mutations, particularly $KRAS^{G13D}$ and $BRAF^{V600E}$, promote the glycolysis rate via increasing glucose uptake through enhancing the expression of GLUT1 (Hutton et al. 2016).

p53 plays an indispensable role in the regulation of various cellular processes, including cell cycle and apoptosis (Zilfou and Lowe 2009; Chen 2016). However, mutated p53 has been observed in various cancers, including CRC (Hollstein et al. 1991). Further, tumor suppressor p53 has also been identified as a key player in the reprogrammed glucose metabolism of CRC (Fang and Fang 2016; Brown et al. 2018). Generally, wild-type p53 attenuates glycolysis via downregulating the expression of GLUT1 and promotes mitochondrial oxidative phosphorylation through suppressing the expression of pyruvate dehydrogenase kinase 2 (PDK2) and enhancing cytochrome c oxidase expression (Brown et al. 2018). Remarkably, loss of p53 in CRC has been found to be linked with suppressed oxidative phosphorylation and increased glycolysis (Brown et al. 2018).

11.2.3 Role of the Glycolytic Nature of Colon Cancer Cells in Chemoresistance

Accumulating reports suggest that reprogrammed glucose metabolism plays a role not only in the survival and metastasis but also in acquiring the chemoresistant property in colorectal cancer cells against several chemotherapeutic agents and thus is also associated with poor prognosis (Zhou et al. 2012). Chemoresistance of colorectal carcinoma against first-line xenobiotic agents appeared as the foremost challenge in the clinical management of CRC. It has been observed that reprogrammed glucose metabolism of neoplastic cells is an essential machinery, which mediates chemoresistivity through its metabolites, including pyruvate and lactate (Lin et al. 2019; Shi et al. 2019; Wang et al. 2018). The increased glycolysis rate acts as a key factor in chemoresistivity of colon cancer through increasing the

intracellular ATP and pyruvate status (Huang et al. 2019). Chemoresistant colon cancer cells need a rapid source of energy for their survival in genotoxic stress, which is fulfilled by their glycolytic nature. Indeed, colorectal cancer patients with poor chemotherapy response showed high expression of glucose transporters, namely, GLUT1, GLUT4, and SGLT1, than chemotherapy-responsive patients (Huang et al. 2019). Further, glycolytic metabolic product pyruvate has been identified as one of the key factors in developing the chemoresistant property in colorectal patients due to its antioxidant property via altering necroptosis and the cell cycle (Huang et al. 2019). Moreover, hyperglycemia or high glucose level has also been reported in inhibiting the 5-FU-induced cell death in four different colon cancer cells, namely, HCT116, SW480, SW620, and LoVo. Thus, the level of glucose contributes a major role in the development of chemoresistance in CRC (Ma et al. 2014). Interestingly, in a study, Zhou et al. have demonstrated that in order to eliminate the xenobiotic stress and acquire the chemoresistant property, colon cancer cells elevate the level of HIF-1α and its downstream molecules, GLUT1 and HKII, for the generation of a high amount of intracellular ATPs through glycolysis (Zhou et al. 2012). Further, in this study, they observed that depletion of intracellular ATP by 3-bromopyruvate treatment resensitized chemoresistant colon cancer cells (namely, HCT116-OxR and HT-29OxR) to 5-FU and oxaliplatin (Zhou et al. 2012). Another study reported that increased LDHA level is associated with 5-FU resistance in colon cancer as inhibition of LDHA by overexpression of miR-34a resensitized resistant colon cancer cells to 5-FU (Li et al. 2015). In a recent study, Wang et al. reported that transient receptor potential canonical channel 5 (TRPC5) acts as an important factor in developing the chemoresistant property to HCT8 and LoVo cells against 5-FU (Wang et al. 2018). TRPC5 is a Ca^{2+} channel which has also been reported in the upregulation of ABCB1 (ATP-binding cassette, subfamily B, member 1) in cancer cells (Ma et al. 2012). Further, in their study, Wang et al. not only found the elevated expression of TRPC5 but also GLUT1 expression in the chemoresistant colorectal cancer cells (namely, HCT8/5-Fu and LoVo/5-Fu cells). Notably, they observed elevated glycolysis in chemoresistant colorectal cancer cells probably due to the reason that the exportation of Ca^{2+} by TRPC5 is an ATP-dependent phenomenon (Wang et al. 2018). A study by Shi et al. showed the role of B7-H3 or CD276, a member of the B7 family of immune regulatory molecules, in CRC chemoresistance (Shi et al. 2019). In this study, they noticed the indispensable role of HKII-mediated glycolysis in B7-H3-induced chemoresistance in CRC. HIF-1α, a central regulator of cancer cell metabolism, is also reported in providing the resistance against anticancer drugs and is associated with poor prognosis in CRC (Baba et al. 2010; Wang et al. 2014). Further, the inhibition of HIF-1α by wogonin through blockage of the PI3K/Akt signaling pathway has been observed in the reversal of the chemoresistant property of HCT116 cells toward anticancer drugs by altering the expression of major glycolysis regulatory molecules like HKII, PDK1, and LDHA (Wang et al. 2014).

Taken together, aforementioned studies suggest the crucial role of aerobic glycolysis in the development of CRC chemoresistance.

11.2.4 Aerobic Glycolysis in the Development of an Efficient Antioxidant System

In cancerous cells, evasion of apoptosis is another imperative hallmark (Hanahan and Weinberg 2011). Cancerous cells acquire the properties of apoptosis evasion by several ways, including developing an efficient antioxidant system to scavenge the reactive oxygen species (ROS). In order to achieve a potent antioxidant system, the glycolytic nature of cancerous cells has been noticed among the critical players, which helps in the production of NADPH via the pentose phosphate pathway (Ghanbari Movahed et al. 2019). Further, few factors like mTOR and YY1 are also reported in the activation of the pentose phosphate pathway and consequently in NADPH production in colon cancer (Ge et al. 2020). The NADPH reduces glutathione (GSH) and thioredoxin, which finally helps in the elimination of reactive oxygen species (ROS) and shields cancerous cells from ROS-mediated cell death due to damage of cellular components, including nucleic acid, lipid, and protein (Alberghina and Gaglio 2014). Further, Kim et al. have observed an increased expression level of glutathione (GSH), GSH synthetase (GSS), and catalytic subunit of glutamate cysteine ligase (GCL) in five different colon cancer cell lines, namely, Caco-2, HCT116, HT-29, SNU-407, and SNU-1033, along with tumor tissues of colon cancer patients (Kim et al. 2015). Glutathione is one of the principal natural intracellular antioxidants composed of glutamic acid, cysteine, and glycine.

Hence, aerobic glycolysis-mediated ROS neutralization may also have a vital role in the development and progression of CRC through developing efficient antioxidant machinery. However, further studies are needed to explore the detailed mechanisms and unidentified pathways through which aerobic glycolysis boosts up the antioxidant system of CRC.

11.2.5 Role of Glycolysis-Induced Acidosis in Colon Cancer Progression

Metabolic reprogramming is one of the striking features, which supports development and progression of colorectal cancer through inducing other crucial hallmarks such as evasion of apoptosis, invasion and metastasis, and immunosuppression (Ehrmann-Josko et al. 2006). The most noticeable changes of CRC cellular bioenergetics include highly elevated glycolysis. The increased glycolysis rate causes the large production of lactate, an end product of aerobic glycolysis. The produced lactate is transported out of the cell through monocarboxylate transporters (MCTs), which leads to acidification of the tumor microenvironment (TME), commonly known as “tumor acidosis” (Pillai et al. 2019). This acidified TME contributes a major role in the CRC progression by augmenting invasion and metastasis, suppressing antitumor immunity, and conferring resistance to chemotherapies (Huang et al. 2019; Koukourakis et al. 2006a).

The acidification of TME induces tumor invasion via increasing the level of extracellular vascular endothelial growth factor A (VEGF-A) and proteases (Koukourakis et al. 2006a). Moreover, elevated lactate level in TME reprograms the metabolic processes of adjacent stromal cells in such a way that they also support tumor growth via supplying metabolic resources to tumor cells. Studies have shown that tumor-secreted lactate is usually taken up by the tumor-associated fibroblasts to utilize as an energy substrate or to generate pyruvate for nearby tumor cells (Koukourakis et al. 2005; Koukourakis et al. 2006b). Thus, CRC-generated lactate helps in the formation of a loop between tumor-associated fibroblasts and CRC which ultimately supports unhindered progression of colorectal cancer. Moreover, lactate-mediated acidosis may provide aggressive behavior to CRC via enhancing properties like evasion of apoptosis, epithelial to mesenchymal transition (EMT), and drug resistance in CRC as in other cancers (Koukourakis et al. 2006a); but still very little is known, and therefore further investigations are needed to identify the responsible mechanistic pathways. In summary, acidosis of TME may also participate in the unhindered development and progression of CRC via modulating apoptosis, invasion and metastasis, and drug resistance.

11.3 Is Glycolysis an Appropriate Therapeutic Target for Colon Cancer Treatment?

The occurrence and progression of colon cancer is a multifaceted process. Tumorigenesis in the colon does not depend on single pathways; rather, it depends on multiple pathways like Wnt, EGFR, and TGF-β signaling pathways (Kinzler and Vogelstein 1996). Further, mutation in APC, p53, Ras, and cMyc genes also contributes to the development of colon cancer (Fearon and Vogelstein 1990). Although colon cancer development is mainly influenced by hereditary components, many cases are periodic and develop sequentially and in a stepwise manner from adenoma to carcinoma (Fearon and Vogelstein 1990; La Vecchia and Sebastian 2020). Further, reprogrammed metabolism has emerged as one of the crucial features of cancer; and, hence, oncologists are focusing on solving the complex maze of metabolic pathways through finding loopholes. These pathways are interconnected to each other, and this has several advantages to cancer cells from initiation to uninterrupted progression until achievement of its immortalization resulting in ultimate death of its host person. However, aerobic glycolysis is considered undeniably one of the central pathways of this complex wiring of metabolic pathways in most of the cancers (Liberti and Locasale 2016; Vander Heiden and Deberardinis 2017; Warburg 1956).

In colon cancer, metabolic reprogramming does occur, and aerobic glycolysis plays a significant role in its progression. Initiation of colon cancer takes place generally through gene mutations that ultimately result in alteration in metabolic pathways either directly or indirectly (Fang and Fang 2016). Studies show that HIF-1α, considered as one of the central players of aerobic glycolysis, plays a critical role in

the tumorigenesis of colorectal cancer (Baba et al. 2010; Santoyo-Ramos et al. 2014; No et al. 2015). Further, reports also suggest that interplay between HIF-1α and APC contributes to colon cancer development where HIF-1α represses the expression of APC (at transcriptional and translational levels), a well-known tumor suppressor gene in several cancers of the GI tract (Nathke and Rocha 2011; Newton et al. 2010). HIF-1α has also been identified as a key mediator molecule in the lysophosphatidic acid (LPA)-mediated colon cancer progression. No et al. have shown that HIF-1α acted as a transcriptional factor for the macrophage migration inhibitory factor (MIF) in LPA-stimulated colon cancer (No et al. 2015). Further, they reported that LPA mediates stabilization of HIF-1α by the formation of a ternary complex of HIF-1α, MIF, and CSN5 under normoxic conditions. In addition, HIF-1α acts as a transcription factor for several glycolysis regulatory molecules such as HKII, PKM2, LDHA, and glucose and lactate transporters such as GLUT1, GLUT3, and MCT4 in several cancers (Masoud and Li 2015; Denko 2008; Semenza 2009). Further, HKII also plays an indispensable role in the progression of colorectal cancer (Liu et al. 2016; Katagiri et al. 2017; Xiong et al. 2017). Katagiri et al. have reported that HKII is associated with tumor size, invasion, and liver metastasis in colorectal cancer (Katagiri et al. 2017). Further, the same group has shown the association of HKII with the recurrence of colorectal cancer and overall survival of colorectal patients. Additionally, they also noticed inhibited proliferation and migration in HCT8 and HT-29 cells transfected with HKII siRNA (Katagiri et al. 2017). Hence, they concluded that HKII could be a potential prognostic marker of colorectal cancer. Xiong et al. have demonstrated the importance of the interplay between pH domain leucine-rich repeat protein phosphatase (PHLPP), a tumor suppressor, and HKII in the regulation of glucose metabolism of colon cancer (Xiong et al. 2017). They observed that PHLPP forms a complex with Akt and HKII and inhibits the glycolysis pathway by attenuating Akt-mediated HKII phosphorylation and subsequently the mitochondrial translocation of HKII (Xiong et al. 2017). Further, glucose transporters also play a significant role in the development and progression of colorectal cancer. Accumulating evidence suggests the significantly elevated expression of GLUT1 in the CRC tumor tissues (Younes et al. 1996; Haber et al. 1998; Merigo et al. 2018). Younes et al. have detected the expression of the GLUT1 protein in 44 colorectal adenocarcinoma tissue samples (83%) out of 53 samples (Younes et al. 1996). In their study, they showed a direct correlation between the expression of GLUT1 and frequency of lymph node metastases in colorectal cancer (Younes et al. 1996). Moreover, Ha and Chi have shown the role of CAV1 (caveolin 1)-mediated GLUT3 expression in the promotion of colon cancer progression through increased glucose uptake and ATP and lactate production (Ha and Chi 2012). There are also few studies which strongly suggest the implication of PKM2 in the colon cancer progression (Yang et al. 2014; Cui and Shi 2015). In a study, Yang et al. have shown that overexpression of PKM2 promotes migration of colon cancer cells via enhancing the expression of migration and invasion regulatory molecules, namely, N-cadherin, Snail-2, active β1-integrin, pFAK, MMP-2, and MMP-9 through the STAT3 signaling pathway (Yang et al. 2014). Further, Cui and Sui have demonstrated the correlation between the upregulated expression of PKM2 and poor prognosis of colorectal cancer (Cui and Shi 2015). Moreover, LDHA is

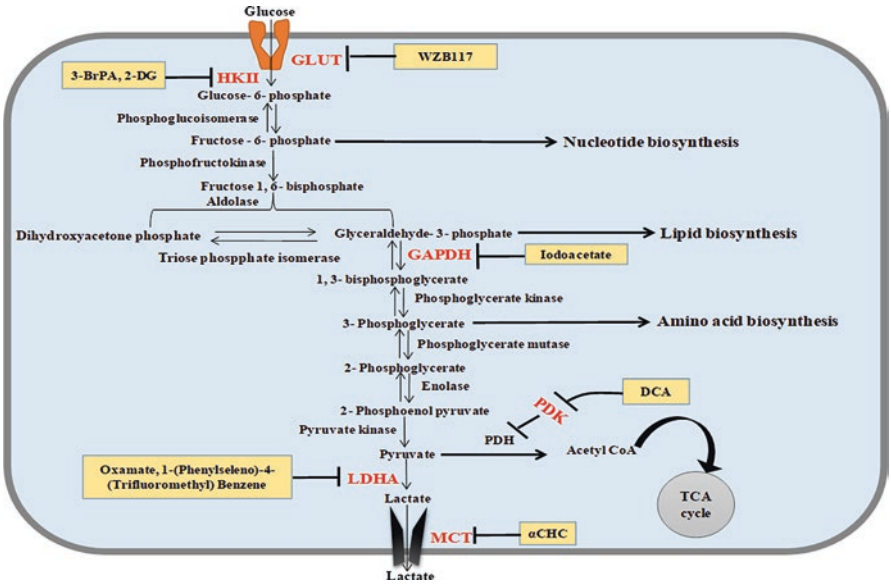

Fig. 11.1 Aerobic glycolysis pathway and probable glycolytic therapeutic targets of colon cancer. Colon cancer cells shift glucose metabolism from mitochondrial oxidative phosphorylation to cytoplasmic glycolysis via blocking the activity of pyruvate dehydrogenase. This metabolic switch supports macromolecular synthesis pathways via supplying precursor molecules and fulfills all the requirements which are needed for unhindered proliferation of colon cancer cells. Therefore, molecules responsible for the altered glucose metabolism could be the potential therapeutic targets for colon cancer

reported in providing the chemoresistant property to colorectal cancer cells against anticancer drugs such as 5-FU. In a study, Li et al. have shown the resensitization of colorectal cancer to 5-FU by miRNA-34a due to suppressed expression of LDHA (Li et al. 2015).

In conclusion, studies illustrate the role of glycolysis regulatory molecules in the regulation and maintenance of colorectal cancer progression. Therefore, glycolysis regulatory molecules could be potential therapeutic targets for colorectal cancer. Figure 11.1 has shown the promising targets of the glycolysis pathway.

11.4 Targeting the Glycolytic Pathway for Colon Cancer Treatment

11.4.1 Glycolytic Inhibitors: Promising Future Therapeutic Options for Clinical Management of Colon Cancer

Colon cancer, being the third most common cancer, employs several mechanisms for its survival and progression. Reprogramming of cellular metabolism has emerged as one of the fundamental characteristics of most cancer cells. Studies during the

last 15 years have evidenced that this metabolic reprogramming is an active process which is governed by oncogenes and tumor suppressor genes and provides cancer cells with energy, reducing equivalents, and biosynthetic precursors (Vander Heiden and Deberardinis 2017). Reprogrammed glucose metabolism is among the most altered metabolic pathways of cancer cells, which is also adopted by the colorectal cancer cells for their uninterrupted growth and development. Therefore, in recent years, in vitro and in vivo tumoricidal activity of various glycolytic inhibitors has been examined against colorectal cancer either alone or in combination with standard chemotherapeutic drugs to develop new therapeutic approaches for the treatment of colon cancer (Sawayama et al. 2015). These inhibitors target key glycolytic enzymes which mainly include HKII, PDK1, and LDHA and transporters of glucose and lactate such as GLUT1, GLUT3, MCT1, and MCT4. WZB117, a GLUT1 inhibitor, has shown an encouraging synergistic effect in reversing the chemoresistance to 5-FU in HT-29 and SW48 cell lines (Liu et al. 2014). Studies have reported HKII as a promising therapeutic target for the treatment of colon cancer. 2DG has been reported in the inhibition of cell cycle, proliferation, migration, and invasion in colon cancer cells in vitro through inhibition of ATP and lactate production (Zhang et al. 2016). Further, 2DG is also reported in the inhibition of B7-H3-induced glycolysis and chemoresistance in colorectal cancer cells (Shi et al. 2019). Another classical inhibitor of HKII, 3-bromopyruvic acid or 3-bromopyruvate (3-BrPA), a bromo-halogenated derivative of pyruvic acid, has exhibited tumoricidal action against colon cancer cells via blocking of ATP production (Scatena et al. 2008; Sun et al. 2015). Further, a study reported cell death in 3-BrPA- or iodoacetate (an inhibitor of GAPDH)-treated colon cancer cells by abolishing the "bioenergetic signature (a ratio of β-F1-ATPase and GAPDH)" (Sanchez-Arago and Cuezva 2011). Interestingly, both of these metabolic inhibitors very effectively augmented tumor regression in a nude mouse transplanted with HCT116 cells compared to the conventional therapeutic drug 5-FU. In addition, α-cyano-4-hydroxycinnamate (α-CHC), an inhibitor of monocarboxylate transporters, has shown the promising antitumor effect against colon cancer cells either in the presence or absence of cisplatin (Kumar et al. 2013b). In this study, α-CHC augmented cisplatin-mediated cell death in Colo205 cells, through altering pH, decreasing glucose uptake and lactate production, and inhibiting glucose metabolism regulatory molecules (Kumar et al. 2013b). Moreover, dichloroacetate (DCA), an inhibitor of PDK, has also exerted antitumor and chemosensitizing action against colon cancer (Liang et al. 2020; Kumar et al. 2013a). Studies by Liang et al. have shown that DCA resensitizes chemoresistant colorectal cancer cells to oxaliplatin and 5-FU by modulating miR-543/PTEN/Akt/mTOR and p53/miR-149-3p/PDK2 pathways, respectively (Liang et al. 2020). Additionally, inhibition of LDHA by oxamate or knockdown has resensitized chemoresistant DLD-1 cells against rapamycin by suppressing glycolysis (Xing et al. 2018). Recently, the research group of Kim et al. has designed a novel LDH inhibitor, 1-(phenylseleno)-4-(trifluoromethyl) benzene (PSTMB), and shown its anticancer activity against colon cancer cells in a dose-dependent manner under normoxic as well as hypoxic conditions through inducing mitochondria-dependent

Table 11.1 List of synthetic glycolytic inhibitors, their targets, and mode of actions

Synthetic compound(s)	Target	Mode of action	Reference(s)
WZB117	GLUT1	Inhibition of glucose uptake	Liu et al. (2014)
2-Deoxyglucose, 3-bromopyruvate (3-BrPA)	HKII	Inhibition of glycolytic influx	Scatena et al. (2008), Sun et al. (2015), Zhang et al. (2016), Shi et al. (2019)
Iodoacetate	GAPDH	Depletion of ATP production	Sanchez-Arago and Cuezva (2011)
Dichloroacetate (DCA)	Pyruvate dehydrogenase kinase	Reactivation of mitochondrial glucose oxidation	Kumar et al. (2013a), Liang et al. (2020)
Oxamate, 1-(phenylseleno)-4-(trifluoromethyl) benzene	Lactate dehydrogenase	Inhibition of glycolytic influx	Kim et al. (2019)
α-Cyano-4-hydroxycinnamate (α-CHC)	Monocarboxylate transporter	Inhibition of lactate export, intracellular acidification, alkalization of tumor microenvironment	Kumar et al. (2013b)

apoptosis via augmenting ROS production (Kim et al. 2019). Table 11.1 has shown the targets and mode of actions of glycolytic inhibitors through which they exert antitumor or chemosensitizing properties in colorectal cancer.

Accumulating reports demonstrate the potential ability of glycolytic inhibitors in the clinical management of colon cancer.

11.4.2 Phytochemicals with Anti-glycolytic Potential in Colon Cancer Treatment

Till date, standard chemotherapy and radiotherapy regimens are among the best therapeutic approaches against colon cancer despite adverse effects. In order to overcome their adverse effects and to achieve high beneficial impact, phytochemicals have emerged as one of the right selections to design novel therapeutic approaches against colon cancer. There are several medicinal plants which have been reported as a great source of chemicals with antineoplastic activity (Sharma et al. 2011). Further, a large number of bioactive phytochemicals have shown very effective antitumor action against several cancers, including colorectal cancer (Sharma et al. 2011). The cytotoxic action of phytochemicals such as curcumin, resveratrol, and sulforaphane has been observed against colon cancer due to

impairment of vital signaling pathways, including MAPK, JNK, Wnt/β-catenin, and AMPK (Yin et al. 2016). Interestingly, the anti-Warburg effect of few phytochemicals has also been reported against colorectal cancer. Therefore, targeting glycolytic activities with bioactive phytochemicals could be a better way to develop effective and safer cancer therapeutic strategies against colorectal cancer.

Wang et al. have reported the anti-glycolytic action of curcumin, a bioactive molecule of the *Curcuma longa* plant (Wang et al. 2015b). In this study, curcumin showed glycolysis inhibitory action in HCT116 and HT-29 cells due to inhibition of expression and activity of HKII. Further, the study also showed curcumin-mediated HKII dissociation from the mitochondrial membrane due to its phosphorylation by Akt. Moreover, a slight decline was also noticed in the expression as well as activity of PFK and LDH.

Atractylenolide 1 (AT-1), a phytochemical isolated from the Chinese medicinal plant Rhizoma *Atractylodes macrocephala*, has shown anti-glycolytic activity against HCT116 and SW480 cells by suppressing the expression of HKII through inhibition of the JAK2/STAT3 signaling pathway (Li et al. 2020).

Avemar, a wheat germ extract, has shown inhibitory action on the expression of PKM2 among various colorectal cancer cell lines such as CACO2, COLO201, DLD-1, WiDr, and LoVo without exhibiting any effect on glycolysis (Shibuya et al. 2015). However, avemar potentially inhibited the pentose phosphate pathway in colorectal cancer cells via downregulating the expression of pentose phosphate pathway enzymes such as glucose-6-phosphate dehydrogenase, transketolase, and phosphogluconate dehydrogenase (Shibuya et al. 2015).

Resveratrol, a polyphenolic molecule of grapes, red wine, and peanuts, shows its tumoricidal activity against various cancers including colorectal cancer (Ko et al. 2017). A study by Fouad et al. has shown the implication of anti-glycolytic activity of resveratrol in the apoptosis induction and attenuation of proliferation and angiogenesis in colon cancer cells (Fouad et al. 2013). Another study by Shibuya et al. has also shown the glycolysis inhibitory ability of resveratrol against colon cancer (Shibuya et al. 2015). Resveratrol has also been reported in the restoration of the normal glucose oxidation process in colon cancer via triggering the oxidative phosphorylation through increasing the activity of pyruvate dehydrogenase complex (PDH) by upregulating the expression of pyruvate dehydrogenase phosphatase 1 (Saunier et al. 2017). The dephosphorylation of phosphorylated pyruvate dehydrogenase by pyruvate dehydrogenase phosphatase (PDP) reactivates pyruvate dehydrogenase and promotes reentry of pyruvate into the TCA cycle for the mitochondrial oxidation (Saunier et al. 2016).

Matrine, a phytochemical isolated from the root of the Chinese plant *Sophora flavescens* Ait, also exhibits anti-glycolytic property. The in vivo and in vitro studies conducted by Hong and Zhong revealed that matrine impedes growth of colon cancer via significantly inhibiting the glucose intake through downregulating the expression of the master transcriptional factor HIF-1α and its downstream targets, namely, GLUT1, HKII, and LDHA (Hong et al. 2019).

Table 11.2 Phytochemicals with glycolysis inhibitory potential

Phytochemical	Source of origin	Affected glycolysis regulatory molecule(s)	Mode of action	Reference(s)
Curcumin	*Curcuma longa*	HKII, PFK, LDHA	Inhibition of glycolytic influx	Wang et al. (2015b)
Atractylenolide	*Atractylodes macrocephala*	HKII	Inhibition of glycolytic influx	Li et al. (2020)
Avemar	*Triticum aestivum*	PKM2	No effect on glycolysis	Shibuya et al. (2015)
Resveratrol	Grapes, berries, peanuts, and red wine	PDP1	Reactivation of mitochondrial glucose oxidation	Saunier et al. (2016)
Matrine	*Sophora flavescens* Ait	HIF-1α, GLUT1, HKII, LDHA	Inhibition of glucose uptake and glycolytic influx	Hong et al. (2019)
Xanthohumol	*Humulus lupulus* L.	HKII	Inhibition of glycolytic influx	Liu et al. (2019)
Dioscin	Various kinds of vegetables and herbs	HKII	Inhibition of glycolytic influx	Zhou et al. (2020)

A polyphenolic compound from *Humulus lupulus*, xanthohumol, exhibits anti-neoplastic action against a wide number of cancers, including colorectal cancer (Zhang et al. 2015; Lee et al. 2007; Monteiro et al. 2008; Jiang et al. 2018). A recent study by Liu et al. showed the role of the glycolysis inhibitory action of xanthohumol behind its anticancer activity against colorectal cancer (Liu et al. 2019). Xanthohumol exhibits anti-glycolytic activity via downregulating HKII expression through inhibition of EGFR-Akt signaling.

Dioscin is a steroid saponin isolated from several plants and has shown its anti-neoplastic activity against various cancers, including colorectal cancer (Yang et al. 2019). In a recent investigation, Zhou et al. have observed in vitro and in vivo glycolysis inhibitory action of dioscin in colorectal cancer cells due to its ability to inhibit HKII expression (Zhou et al. 2020).

Overall, above-mentioned reports suggest that phytochemicals with anti-glycolytic activity could have a great potential to be utilized as therapeutic agents against colorectal cancer. Table 11.2 presents the overview of anti-glycolytic actions of above-discussed phytochemicals.

11.5 Conclusion and Future Perspectives

Colon cancer is one of the leading causes of cancer-associated deaths worldwide. Despite advancement in technologies and development of new therapeutic approaches, the clinical management of colon cancer patients is still a big

challenge. The adverse effects of chemotherapy and colon cancer chemoresistance are the major factors associated with the poor survival of colon cancer patients. The chemotherapy-associated problems can be overcome by targeting molecules which mediates colon cancer progression in a multifaceted manner. The identification of such targets may help in the development of effective and safer chemotherapeutic strategies for the treatment of colon cancer. Interestingly, several recent studies strongly suggest the crucial role of aerobic glycolysis in the development and progression of colon cancer, and therefore targeting of abnormally activated/expressed glycolysis regulatory molecules by their inhibitors/antagonists/siRNAs may help in designing novel therapeutic regimens for colon cancer.

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Chapter 12
An Insight into the Therapeutic Potential of Phytochemicals for Colorectal Cancer: Latest Perspective

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Abstract The adenomatous polyps formed from the inner lining of the colon and rectum generally transform into cancer, and this makes up to 96% of colorectal cancer formations. There are local and systemic therapies available as per the stage of cancer. But secondary complication associated with these drugs is the major drawback associated with these. The plants because of their biodiversity are rich in phytochemicals that have therapeutic applications. Numerous phytochemicals exhibit anticancer activity. This chapter reviews the various phytochemicals that show higher efficiency and efficacy in the treatment of colorectal cancer with special emphasis on their mechanism of action.

Keywords Colorectal cancer · Phytochemicals · Molecular mechanisms · Antiproliferative · Wnt/β-catenin signalling

12.1 Introduction

The present-day statistics define colorectal cancer (CRC) as the second most common type of cancer and is the fourth leading cause of cancer-related deaths worldwide because of its late diagnosis (Koveitypour et al. 2019; Wan et al. 2020). It is among the top three cancers in men following lung and prostate cancers with an incidence rate of 920,000 cases and mortality rate of 456,000 cases. CRC ranked as

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the second most common cancer after breast cancer in women with 733,000 cases of incidence and 376,000 deaths (Fitzmaurice et al. 2019). It is a heterogeneous disease more prone with age and is defined by genetic predisposition and environmental factors, thus leading to multiple genetic and epigenetic alterations in vital genes. The specific factors that pose more risk in the process are intestinal commensals and pathogens, a diet high in fat and low in fibre, a sedentary lifestyle, obesity, cigarette smoking, alcohol abuse, etc. (Le Marchand et al. 1997; Colussi et al. 2013). These factors apparently induce inflammation. Chronic inflammation is reported as one of the leading causes for the induction and progression of cancer.

Chronic inflammation induces the constant expression of various cytokines and growth factors that promote uncontrolled cell division. It is also equally responsible for the free radical generation that causes genetic and epigenetic alterations in oncogenes and tumour suppressor genes leading to malignancy. The genetic and epigenetic alterations developed will lead to the transformation of normal mucosa to adenoma and then to carcinoma through three diverse pathways like chromosomal instability (CIN) pathway (Pino and Chung 2010), microsatellite instability (MSI) pathway (Boland and Goel 2010) and the CpG island methylator phenotype (CIMP) pathway (Rhee et al. 2017).

The gain or loss of large fragments of DNA ranging from chromatids to chromosome is observed in chromosomal instability pathway, thus leading to aneuploidy, telomere dysfunction or defects in the DNA damage response mechanisms. CIN is mostly observed in sporadic cases than inhereditary cases of CRC. In the microsatellite instability pathway, the alterations in the microsatellite sequences are predominantly caused due to the repetitive nature of the microsatellites marking for frameshift mutations. These mutations are normally corrected by DNA mismatch repair (MMR) system. However, the failure of the MMR system could lead to mutations in the tumour suppressor genes and oncogenes leading to malignancy. MSI pathway plays a crucial role in hereditary CRC followed by sporadic CRC. The CIMP pathway is characterised by hypermethylation or histone modifications in the promoter regions of tumour suppressor genes or tumour-related genes leading to their inactivation. This pathway is predominantly observed in sporadic CRC, but all the pathways are not mutually exclusive (Al-Sohaily et al. 2012; Nguyen and Nguyen 2018). All these three pathways create aberrations in the oncogenes and tumour suppressor genes leading to dysregulation of signalling pathways majorly involved in cell development, differentiation, proliferation, apoptosis, etc. Some of the pathways that are primarily reported are the epidermal growth factor receptor (EGFR), PI3K/AKT, Notch, transforming growth factor-β (TGF-β) and Wnt signalling pathways (Colussi et al. 2013; Koveitypour et al. 2019; Wan et al. 2020). The CRC is a progressive disease that has the involvement of more than one pathway. Crosstalk between different pathways is also reported (Koveitypour et al. 2019; Wan et al. 2020). The aberrant expression of receptors, ligands and downstream targets of these pathways in CRC cells has been confirmed by many studies. Therefore, one of the therapeutic approaches for CRC is the regulation of such genes through different drugs or compounds. The current therapeutics include drugs like antimetabolites (e.g. methotrexate), antitubulin agents (e.g. Taxanes),

DNA-intercalating agents (e.g. Cisplatin, doxorubicin), hormones etc., which have several side effects. These drugs create several physiological to phenotypic changes like gastrointestinal lesions, neurologic dysfunction, suppression of bone marrow, drug resistance, hair loss, hyperpigmentation, etc. Therefore, the search for molecules with better anticancer activity and lesser side effects still prevails.

From time immemorial, natural products have played a significant role in human health (Cragg and Pezzuto 2016). A number of plants used in traditional medicine have been found to possess antiproliferative and tumour suppressor activities. The complementary and alternative medicines have gained attention in curing several diseases using plant-based extracts (Pan et al. 2013). In the current scenario, there is a compulsive and urgent need to develop anticancer compounds without much complexity. The plant-based compounds have minimal side effects than chemically synthesised compounds and are used from time immemorial (Veeresham 2012). This chapter presents an update on some of the prominent dietary phytochemicals that are in regular usage with proven anti-CRC effects.

12.2 Curcumin

Curcumin (1,7-bis(4-hydroxyl-3-methoxyphenyl)-1,6-heptadiene-3,5-dione), also called diferuloylmethane, is a phytochemical with a molecular weight of 368.4 g/mol and melting point of 183.0 °C, and the chemical formula is $C_{21}H_{20}O_6$ (Fig. 12.1) (Farazuddin et al. 2014).

The curcumin, a phytochemical derived from turmeric (*Curcuma longa*), which is a plant similar to ginger, belongs to the family Zingiberaceae (Shehzad et al. 2010). Chemically, it is a natural phenol with a typical yellow colour. It is easily soluble in ketone, acetic acid, alkali and chloroform, while it is insoluble in water at acidic and neutral pH (Chattopadhyay et al. 2003). Due to its hydrophobic properties, curcumin is able to diffuse through cell membranes into the mitochondria, endoplasmic reticulum and nucleus; in all these sites, it can exert its action (Jaruga et al. 1998).

Curcuma longa, a medicinal herb, has been traditionally used in Asian countries due to its antioxidant, anti-inflammatory (Lestari and Indrayanto 2014), antimutagenic, antimicrobial (Mahady et al. 2002; Reddy et al. 2005) and anticancer properties (Vera-Ramirez et al. 2013; Wright et al. 2013). Indeed, it has been suggested as an anti-carcinogenetic agent for several tumours, including prostate, pancreas,

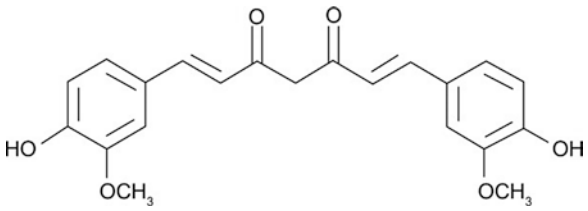


Fig. 12.1 Chemical structure of curcumin

breast, stomach, liver carcinomas and leukaemia (Hanif et al. 1997; Aggarwal et al. 2003; Lopez-Lazaro 2008). Among the proposed mechanisms of action, initiation of epithelial apoptosis appears to be the most investigated (Ismail et al. 2019). Indeed, it has been shown that the curcumin can promote the synthesis of proteins related to apoptotic processes and could interplay with the pathways of inflammation-related programmed death (Plummer et al. 1999; Bush et al. 2001; Agarwal et al. 2018; Su et al. 2018).

Curcumin interferes with multiple cellular signalling pathways such as Wnt/β--catenin signalling, phosphoinositide 3-kinase (PI3K)/protein kinase B (Akt) pathway, Janus kinase (JAK)/signal transducer and activator of transcription (STAT) signalling pathway, mitogen-activated protein kinase (MAPK) pathway and p53 signalling and nuclear factor-κB (NF-κB) pathway (Kunnumakkara et al. 2017). Curcumin is capable of altering multiple cellular targets and signalling pathways that are involved in cancer cell growth, apoptosis, invasion and metastasis.

The Wnt/β-catenin signalling is an evolutionarily conserved pathway and is implicated in the pathogenesis of various human diseases. A study on human colon cancer cells showed that curcumin inhibited the Wnt/β-catenin pathway by suppressing c-myc expression and instigating caspase-3-mediated cleavage of beta-catenin, E-cadherin and APC (Narayan 2004).

Curcumin inhibits cell proliferation by cell cycle arrest at the G2/M phase and partially at the G1 phase in the human colon cancer cell line HCT116 (Mosieniak et al. 2012). Curcumin negatively regulates cyclin D1 and induces cell cycle interruption at the G1 phase in the same colon cancer cell line HCT116 (Lim et al. 2014). Cyclin D1 binds to both CDK4 and CDK6 and forms an active complex phosphorylating Rb protein at serine 780, thereby regulating the transition from G1 to S phase (Hernando et al. 2004). Curcumin also inhibits the proliferation of colon cancer cell lines by the induction of reactive oxygen species generation and downregulating the E2F4 and related genes such as cyclin A, p21 and p27 (Kim and Lee 2010).

One of the important mechanisms through which curcumin blocks cell growth is by the induction of apoptosis. This process in the colorectal cancer cells involves multiple molecular targets including enzymes (cyclooxygenase-2 (COX-2)), transcription factors (NF-κB and beta-catenin), Bcl-2 family members (Bcl-2, Bax and Bcl-xL), death receptors (death receptor 5 (DR5) and Fas), protease enzymes (caspase-3 and caspase-8) and reactive oxygen species (Pricci et al. 2020). Curcumin was shown to downregulate COX-2 gene expression in colorectal cancer cells (Goel et al. 2001; Yin et al. 2016).

Curcumin also exhibits its action on colorectal cancer cells by targeting the colorectal stem cells (CSCs). Cancer cells acquire stemness and drug resistance properties by the activation of the Wnt/β-catenin, Notch and SHH pathways. Curcumin effectively inhibits the activation of these pathways at the receptor by (1) inhibition of the ligand-binding site of the receptor, (2) inhibition of the formation of the receptor complex (3) and/or reduction in the abundance of the receptor. Curcumin reduces the expression levels of the downstream effectors of these pathways at the mRNA and protein levels (Ramasamy et al. 2015).

In short, curcumin modulates the fate of cancer stem cell by targeting misregulated signalling pathways at multiple cellular levels, namely, receptors and downstream effectors, and transcriptional activity in Hedgehog, Notch, PI3K/Akt/mTOR and Wnt/β-catenin signalling pathways.

MicroRNA (miRNA) regulates a variety of biological events, including development, cell proliferation, differentiation, senescence and apoptosis. The two downstream targets of miR-22, SP1 transcription factor (SP1) and oestrogen receptor 1 (ESR1), have been shown to promote tumour development. Overexpression of SP1 promotes metastasis of different tumours. The treatment of the cells with curcumin suppresses CSC growth and induces apoptosis. Curcumin inhibits the transcriptional regulation of miR-21 via AP-1; suppresses cancer cell proliferation, invasion and metastasis; and stabilises the expression of the tumour suppressor programmed cell death protein 4 (Pdcd4) (Mudduluru et al. 2011). Curcumin also enhances the sensitivity of cancer cells to anticancer drugs in addition to playing a role in reversing epithelial-mesenchymal transition (Ramasamy et al. 2015). Curcumin also plays an important role in upregulating the tumour-suppressive miRNAs such as Let-7, miR-26a, miR-101 and miR-146 (Li et al. 2009; Bao et al. 2011).

12.2.1 Gallic Acid

Gallic acid (GA) (3,4,5-trihydroxybenzoic acid) is a phenolic compound with molecular weight 170.12 g/mol and molecular formula $C_7H_6O_5$ or C_6H_2 $(OH)_3$ COOH (Fig. 12.2). It forms white, yellowish-white or pale fawn-coloured crystals of organic acid. The molecule is soluble in alcohol and ether, but it has low solubility in water. The melting point of gallic acid is 250 °C, and above this temperature, it is converted into carbon dioxide and pyrogallol (Goldberg and Rokem 2009).

Gallic acid is found both in free state and as a constituent of tannins, namely, gallotannin. The gallic acid and its derivatives are present in every part of the higher plants such as bark, wood, leaf, fruit, root and seed (Daglia et al. 2014). It occurs in clove buds (*Eugenia caryophyllata* Thunb.) (175 mg/kg), green tea (6 mg/kg − 1) and red wine (8–71 mg/L). The primary dietary sources include red fruits such as strawberries, raspberries and blueberries, black tea, red wine and nuts (Crozier et al. 2006a).

The anticancer activities of gallic acid are the prevention of cellular proliferation, promotion and generation of reactive oxygen species (ROS) and cell cycle arrest in G2/M.

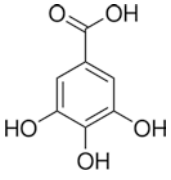


Fig. 12.2 Chemical structure of gallic acid

GA inhibits transcription factors AP-1, NF-κB, STAT1 and OCT-1 expression in Caco-2 cells and suppresses Wnt/β-catenin signalling in HCT116 cells (Lee et al. 2016). Gallic acid shows its effect on colorectal cancer by arresting cell cycle at G0/G1 phase through the decrease of cyclin D1 level, also by inducing apoptosis by activating caspase-3 expression in Caco-2 cells (Forester et al. 2014) and by increasing ROS generation and decreasing MMP in HCT-15 cells (Subramanian et al. 2016).

A study by Subramanian et al. (2016) showed that the phenolic compound GA exhibits an antiproliferative effect on the HCT-15 colon cancer cell lines in a dose-dependent manner. Increasing concentrations of GA leads to lysis of human colon tumour (HCT-15) cells with either by necrosis or apoptosis; as a result, cell growth inhibits. Gallic acid influences the colony formation of HCT-15 cells. Colon cancer cells subjected to gallic acid show changes in morphology such as membrane blebbing and cell shrinkage.

GA initiates a natural apoptotic pathway through the caspase-9 application and PARP cleavage in CT-26 and HCT116 cells, and it also stimulates cell cycle arrest in the G1 stage via p53 activation (Samad and Javed 2018). GA induces the differentiation of CSCs and self-renewal capacity through downregulation of the expression of the CSC markers, CD133, CD44, DLK1 and Notch1 in HCT116 cancer cells (Lee et al. 2016).

A study by Zhao et al. (2013) reported that the fermented Pu-erh tea showed more potent anticancer activity than the unfermented Pu-erh tea and green tea due to increased GA in HT-29 cells. Giftson et al. (2010) reported that due to its phenolic group, GA could inhibit lipid peroxidation, decrease lipid peroxidation products and also deplete the levels of antioxidants in colorectal cancer. It also suppresses reactive oxygen species and enhances the level of GSH.

Epigallocatechin-3-gallate (EGCG) (ester of epigallocatechin and gallic acid) when used in combination with 3,4-dihydroxyphenylacetic acid (3,4-DHPAA) notably increased the antiproliferative activity in vitro in HCT116 colon cancer cells compared to EGCG or DHPAA when used alone (Henning et al. 2013). Pandurangan et al. (2015) demonstrated that in chemically induced colitis in mice, GA suppressed the level of expression of inflammatory markers such as IL-6, COX-2 and iNOS and the degradation of the inhibitory protein IκB. The study also showed that GA reduced the activation and nuclear accumulation of p65-NF-κB and p-STAT3Y705 in colonic mucosa.

In addition, GA was reported to activate p53 upregulated modulator of apoptosis (in HCT116 cells), which is a pro-apoptotic protein that increases the release of cytochrome *c* from the mitochondria through disruption of the mitochondrial membrane potential (MMP), which demonstrates the participation of the intrinsic apoptosis pathway (Yang et al. 2018).

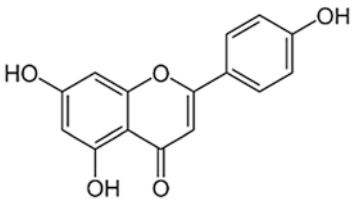


Fig. 12.3 Chemical structure of apigenin

12.3 Apigenin

Apigenin (4′,5,7-trihydroxyflavone) is a flavone under the category of natural flavonoid. Apigenin is a plant-derived flavonoid with a molecular weight of 270.24 g/mol and molecular formula $C_{15}H_{10}O_5$ (Fig.12.3).

Apigenin is a widely distributed flavone available in fruits and vegetables, such as parsley, Chinese cabbage, bell pepper, garlic, celery and guava (Manach et al. 2004a, 2004b). Apigenin exhibits strong cytostatic and anti-angiogenic effects in vitro (Hirano et al. 1989; Engelmann et al. 2002). Apigenin induces growth inhibition, cell cycle arrest and apoptosis in colorectal cancer cells (Zhong et al. 2010; Lee et al. 2014; Yang et al. 2015).

Apigenin has been exhibited to be an effective agent for triggering apoptosis through either the intrinsic or extrinsic pathway in human cancer cells. Apigenin upregulates Bim expression and downregulates Mcl-1 expression, thereby synergising with the Bcl-2 inhibitor ABT-263 to trigger mitochondria-dependent cell apoptosis. The induction of apoptosis by apigenin might be related to its ability to its pro-oxidative effect, leading to increased ROS production and oxidative stress (Banerjee and Mandal 2015). Apigenin also promotes apoptosis by inducing the expression of p53 and altering the Bax/Bcl2 ratios (Zhong et al. 2010; Chidambara Murthy et al. 2012). Apigenin treatment potently inhibits cell growth by the induction of cell arrest at G2/M phase that is associated with suppression of cyclin B1 and its activating partners, Cdc2 and Cdc25c, and increase of cell cycle inhibitors, p53 and $p21^{WAF1/CIP1}$, in human colorectal carcinoma HCT116 cells (Lee et al. 2014). Apigenin can induce G2/M cell cycle arrest of multiple colon cancer cell lines including SW480, HCT116, HT-29 and Caco-2 to varying degrees, which was associated with decreased expression of cyclin B1 proteins and the cyclin-dependent kinase p34(cdc2) (Wang et al. 2000; Wang et al. 2004; Chung et al. 2007; Lee et al. 2014).

In colorectal cancer cell lines, DLD1 and SW480, apigenin inhibits cell migration, invasion and metastasis through modulating the NEDD9/Src/AKT cascade (Dai et al. 2016). Apigenin also prevents cell proliferation and migration by upregulating transgelin and downregulating MMP-9 expression by decreasing the phosphorylation of AKT, thereby inhibiting tumour growth and metastasis to the liver and lung (Chunhua et al. 2013).

Apigenin enhances ABT-263-induced anti-tumour activity via the inhibition of the pro-survival regulators AKT and ERK in vitro and in vivo in the colorectal cancer cell lines HCT116 and DLD1 (Shao et al. 2013). There is an increased expression of Wnt, frizzle or lymphoid enhancer factor (LEF)/T cell factor (TCF) in Wnt/β-catenin signalling pathway (Giles et al. 2003; Krausova and Korinek 2014; Ahmadzadeh et al. 2016; Pohl et al. 2017). Apigenin significantly inhibits the Wnt/β-catenin signalling pathway and thus suppresses cell proliferation, migration and invasion in colorectal cancer (Liu et al. 2015; Xu et al. 2016).

Apigenin downregulates total, cytoplasmic and nuclear β-catenin through the induction of the autophagy-lysosomal system. The auto-lysosomal degradation of β-catenin by apigenin occurs via inhibition of the AKT/mTOR signalling pathway (Lin et al. 2017).

Inflammatory bowel disease and colitis-associated colon cancer in mice are implicated with reduced NF-κB activation and STAT3 activation. Apigenin induces NLRP6 expression within the intestinal epithelium. NLRP6 is shown to be important for regulating the composition of gut microbiota and for protection against the development of colitis and inflammation-associated tumorigenesis (Elinav et al. 2011; Normand et al. 2011; Chen et al. 2011; Levy et al. 2015; Seregin et al. 2017).

12.4 Ellagic Acid

Ellagic acid (EA) (2,3,7,8-tetrahydroxy-chromeno) is a polyphenolic compound with molecular weight 302.197 g/mol and chemical formula $C_{14}H_6O_8$ (Fig. 12.4). It is highly thermostable with a melting point of 350 °C. EA contains four phenolic rings that are electron acceptors and represent the hydrophilic domain (Sepulveda et al. 2011). EA is abundantly found in berries, nuts, grapes, etc. (Girish and Pradhan 2008). Berries on average contain 47–90 mg/g of EA, particularly blackberries (88 mg/kg), strawberries (18 mg/kg) and raspberries (5.8 mg/kg) (Wada and Ou 2002, Crozier et al. 2006a, 2006b). Persian walnut and chestnut contain 9.3 mg/kg and 7350.44 mg/kg of EA, respectively (Li et al. 2006; De Vasconcelos et al. 2007). Pomegranate contains a high concentration of ellagitannins which will be hydrolysed to EA, thus becoming one of the richest and easily available sources of EA (Heber 2008).

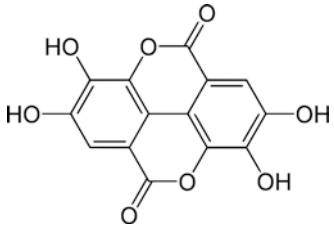


Fig. 12.4 Chemical structure of ellagic acid

EA is indicated with multiple therapeutic activities like anti-atherosclerotic, anti-cancer, anti-obesity, etc. It possesses preventive and therapeutic effects against various cancers, including colorectal cancer (Heber 2008, Yousef et al. 2016a, 2016b). Several studies provided evidence of the vital activity of EA in the prevention of cardiovascular diseases and degenerative diseases like cancer due to its antioxidant properties (Manach et al. 2004a, 2004b). Additionally, EA also possesses antimutagenic, antibacterial, anti-inflammatory, antiviral, antidiabetic, hepatoprotective, antifungal, neuroprotective, antihyperlipidemic, gastroprotective and antidepressant properties (Evtyugin et al. 2020). Before 2004, there were no reports on the bioavailability of EA in the human body. Seeram et al. conducted in vivo experiments, where 180 ml of pomegranate juice containing 25 mg EA and 318 mg of ellagitannins were used for experimentation. The maximum concentration of 31.9 ng/ml of EA was detected in human plasma after 1 h ingestion and was completely eliminated by 4 h, proving that EA could also be a reliable biomarker for human bioavailability studies (Seeram et al. 2004).

EA was evaluated for its antiproliferative activity on HT-29, HCT116, SW480 and SW620 colon tumour cells at 12.5–100μg/ml. The cell proliferation was inhibited in a dose-dependent manner in all cell lines with the trend as HCT116 > SW480 > SW620 > HT-29. The EA exhibited apoptotic and antioxidant activities when tested against HT-29 and HCT116 cells (Seeram et al. 2005). Equally, EA was studied for its chemosensitivity effects on human colorectal carcinoma cells to 5-fluorouracil. EA and 5-fluorouracil alone and in combination were tested on multiple colorectal cancer cell lines, viz. Colo 320 DM, HT-29, LoVo and SW480 cells at a concentration range of 2.5–25μg/ml and 5–25μM, respectively. EA and 5-fluorouracil synergistically decreased the cell proliferation of Colo 320DM, SW480 and HT-29 cells by inducing apoptotic cell death. The ratio of BAX:BCL-2 protein was enhanced, which triggered the caspase-3 activation leading to the apoptosis of the cell lines; thus, EA potentiates chemosensitivity of HT-29, Colo 320DM and SW480 CRC cells to 5-FU (Kao et al. 2012).

Further, in another study, HCT116 and Caco-2 cells were treated with EA where suppression of Akt phosphorylation was observed in both the cell lines. EA indicated its antiproliferative effects on other CRC cell types also where it showed effect by suppressing the cell proliferation through cell cycle arrest by activating caspase-8, translocated Bax to the mitochondrial fraction of cells and reduced PCNA expression. EA inhibition of colon cancer HCT116 cells is through simultaneous regulation of expression of colon cancer target genes, including CCNB1, PRKACB, JUN, CDC20, MEF2C and IL8. Further, EA has been confirmed to downregulate protein kinase C (PKC), which is vital for cell proliferation (Mishra and Vinayak 2013). EA induces novel and a typical PKC isoform and produces caspase-3-dependent apoptosis by blocking energy metabolism. EA can hinder colorectal tumorigenesis (Mirsane and Mirsane 2017, Yousef et al. 2016a, 2016b).

Kyoto Encyclopedia of Genes and Genomes (KEGG) and Gene Ontology pathway analysis showed that numerous cellular functions were changed by EA including proliferation, cell cycle, apoptosis and angiogenesis. In HCT cell line, EA decreased levels of cyclin B1 in CRC cells, which could block G2/M transition and

several steps in mitosis, if initiated (Zhao et al. 2017). EA drastically decreased a series of VEGF-induced angiogenesis processes including migration, proliferation and tube formation of endothelial cells. Moreover, it directly hindered its downstream signalling pathways, including PI3K/AKT and MAPK in endothelial cells and VEGFR-2 tyrosine kinase activity (Wang et al. 2012). EA can also mitigate the invasive potential of tumours through the regulation of protease activity (P Pitchakarn et al. 2013). EA can also minimise the cancer cell viability by elevating the caspase-3 activity, reducing the activity of telomerase and downregulating Bcl-2 (ST Huang et al. 2009). Another study also indicated that PI3/Akt kinases were inactivated by EA, and it helped inducing apoptosis through the caspase-3 pathway with the involvement of Bcl-2, Bax and cyt c in DMH-induced rat colon carcinogenesis (Umesalma and Sudhandiran 2011). EA has suppressed proliferation of multiple cell lines at concentrations ranging 2.5–25μg/ml by suppressing or downregulating MAPK and PI3K pathways.

12.5 Sulforaphane

Sulforaphane (SFN) (1-isothiocyanato-4-(methanesulfinyl)butane) is a compound which belongs to the isothiocyanate group of organosulfur compounds with molecular weight 177.29 g/mol and chemical formula $C_6H_{11}NOS_2$ (Fig.12.5).The melting point of SFN is 74.6 °C. The major sources of SFN are cauliflower, cabbage, broccoli and other cruciferous vegetables. It is found mostly in cruciferous vegetables (Frydoonfar et al. 2004).Higher levels of sulforaphane is found in fresh cabbage (540μg g^{-1}), broccoli (220μg g^{-1}) and Brussels sprout (120μg g^{-1}) (Mohamed et al. 2010). SFN possesses potent anticancer and anti-inflammation properties (Woo and Kwon 2007). The compounds containing electrophilic carbon structures (R–N=C=S) in general have stronger antiproliferative activity. The significant advantage for SFN is its higher bioavailability, structure and lipophilicity (Houghton 2019).

In an in vitro study, CRC cells were treated with SFN in increasing doses (0, 6.25, 12.5, 25 and 50μM) for 24 hrs. It was observed that the proliferation and survival of the primary colon cancer cells declined in a dose-dependent manner. A significant morphological change was observed in the primary colon cells, and SFN also induced cell death. SFN hindered tumour growth by arresting the cell cycle at G2/M phase and by inducing caspase and mitochondrial pathway of apoptosis. SFN could remarkably induce the caspase-3 activity of colorectal cancer cell lines (Chen et al. 2012a, 2012b). SFN was found to block phosphorylation of ERK and AKT and activated FOXO transcription factors, which lead to cycle arrest and cell apoptosis (Roy et al. 2010).

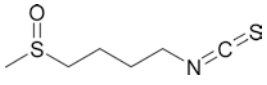


Fig. 12.5 Chemical structure of sulforaphane

SFN decreased the growth of SW480, DLD1 and HCT116 colorectal cell lines via inhibition of proliferation and activation of cell death. Interestingly, in colorectal cancer cells, SFN inhibits Wnt/β-catenin signalling, which occurred by the suppression of β-catenin target genes (AXIN2, LGR5) and by activating the β-catenin destruction complex pathway downstream (Bernkopf et al. 2018).

SFN prevented the growth of colon cancer cell lines HT-29, SNU-1040, DLD11 and HCT116, by inducing apoptosis and G2/M phase cell cycle arrest through the regulation of the P38 and JNK pathways (Byun et al. 2016). SFN prevented hypoxia-induced HIF-1α expression in AGS human gastric cancer cells and HCT116 human colon cells; it also inhibited hypoxia-induced VEGF expression in HCT116 cells; it even hindered hypoxia-related target protein expressions like VEGF, glucose transporter 1 (GLUT1) and heme oxygenase (HO)-1 (Kim et al. 2015). Multiple research studies have proved the effect of SFN on HCT116 cells.

SFN was studied for its sensitisation of oxaliplatin (Ox)-treated CRC cells, viz. Caco-2 and SW620 cells, where it was observed SFN and Ox prevented cell growth in a dose-dependent manner both individually and synergistically. Co-treated cells further indicated various morphological changes that occurred throughout the apoptotic process, like cell surface exposure of phosphatidylserine, cytoplasmic histone-related DNA fragments and membrane blebbing. Further, both intrinsic and extrinsic apoptotic pathways were observed. Prolonged treatments with higher conentrations exhibited necrosis in the cell lines. It is further proved that the SFN could sensitise Ox-treated colon cancer cells by prevention of cell growth through the induction of various modes of cell death (BM Kaminski et al. 2011). SFN showed outstanding cytoprotective properties in untransformed colon epithelial cells, and it caused Nrf2 translocation and induction of its target genes like NQO1, MRP, etc. a significant detoxifying and transport proteins.

In a study on HT-29 and Caco-2 cells, the SFN induced Nrf2 target enzyme activity differently where the level of NQO1 was higher in HT-29 and MRP2 was higher in Caco-2 cells. In HT-29 cells, SFN brought a cellular response that is beneficial in the NQO1-mediated bioactivation of cytostatic prodrugs and Caco-2 cells, and SFN escalated transport protein activity; it could weaken the chemotherapy efficacy (Lubelska et al. 2016). In all the other CRC cells except Caco-2, the SFN has depicted anticancer activity. SFN also suppressed the growth of colon cancer cell lines HT-29, SNU-1040, KM12 and DLD1 by arresting G2/M phase transition of cell cycle and inducing apoptosis, concomitant with phosphorylation of CDK1 and CDC25B at inhibitory sites, and upregulation of the p38 and JNK pathways (Byun et al. 2016). In p53-deficient SW480 cells, SFN-induced mitochondria-associated cell apoptosis, concomitantly by disruption of the mitochondrial membrane potential, enhanced Bax/Bcl-2 ratio, as well as activation of caspase-3, caspase-7 and caspase-9. Further, it is also associated with enhanced reactive oxygen species (ROS) production and the activation of extracellular signal-regulated kinases (Erk) and p38 mitogen-activated protein kinases (Lan et al. 2017).

SFN synthesised thiourea-functionalised silicon nanoparticles (SiNPs) exhibited anticancer activity. This study indicated the decrease of over expressed EGFR inCaco-2 cells (Behray et al. 2016). SFN repressed proliferation of different CRC

cell lines by the regulation of caspase and mitochondrial pathway, β-catenin pathway and P38 and JNK pathways.

12.6 Allicin

Allicin (S-prop-2-en-1-yl prop-2-ene-1-sulfinothioate) is a major bioactive compound in the extract of garlic (*Allium sativum* L.) with a molecular weight of 162.28 g/mol, and its molecular formula is $C_6H_{10}OS_2$ (Fig. 12.6). The melting point of allicin is 25 °C. It is an organosulfur compound, which is produced from the non-proteinogenic amino acid alliin (S-allylcysteine sulfoxide) by the activity of alliinase when the garlic is chopped or crushed (Bayan et al. 2014). In a fresh garlic bulb, allicin amounts 1500–27,800 ppm (Omar and Al-Wabel 2010). Allicin is reported to have numerous biological activities such as anti-protozoal, antimicrobial, antifungal, antiviral, antioxidant, anti-inflammatory, anticancer, anti-Alzheimer's, antidiabetic and antihypertensive activities, immunomodulatory effect, etc. (Batiha et al. 2020).

Clinical studies indicated that allicin has anticancer and tumour suppressive properties. The induction of apoptosis was vital for the anticancer effect of allicin, either by a caspase-dependent or caspase-independent mechanism. In addition to caspase activity, apoptosis-inducing factor (AIF) is also said to play crucial in the allicin-induced cell death (J Borlinghaus et al. 2014, Zhang et al. 2015). Allicin inhibits the proliferation and induces apoptosis in the MGC-803 human gastric carcinoma cells through the p38 mitogen-activated protein kinase/caspase-3 signalling pathway (Zhang et al. 2015). Allicin suppressed the cell proliferation of colon cancer cell lines HCT116, LS174T, HT-29 and Caco-2 in time and dose-dependent manner at concentrations ranging from 6.2 to 310μM. Allicin was shown to alter Bax/Bcl2 ratios leading to the disruption of mitochondrial membrane potential and release of cytochrome *c*, thereby inducing apoptosis. This mechanism is associated with the transactivation of the transcription factor Nrf2 (Chen et al. 2010).

In vivo studies on AOM/DSS mice have proved the antiproliferative property of allicin where there is a significant decrease in the number and size of tumours. In the same study, the in vitro studies on HCT116 cells substantiated the antiproliferative role of allicin. It is reported that the concentration of phosphorylated STAT3 decreased in a dose-dependent manner of allicin treatment, thereby leading to antiproliferative and apoptotic activities. These results suggest that allicin induces apoptosis and inhibits proliferation of cancer cells via STAT3 signalling pathway also (Li et al. 2019).

Allicin increased hypodiploid DNA content and enhanced capability of producing cytochrome C from mitochondria to cytosol in colon cancer, and in result, it

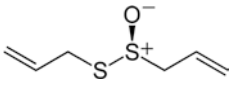


Fig. 12.6 Chemical structure of allicin

showed apoptotic cell death (Zou et al. 2016). Allicin showed significant inhibition on colon cancer LoVo cell line; it could repress the telomerase activity and expression of protein and mRNA and nuclear factor-kappa B (Guang et al. 2004). Kim and co-workers examined the antiproliferative and anticancer activity of allicin and identified modifications by allicin treatment in human colorectal HCT116 cells. Allicin reduced cell viabilities in time and dose-dependent manner and induced apoptosis. With the DNA microarray analysis, they showed that allicin significantly induced NAG-1 and ATF3 gene expression. Expression of NAG-1 protein was dependent on p53 presence (Kim et al. 2010). In a study, Caco-2 cells were treated with allicin, and it showed a drastic increase of catalase in these cells (Mahdy et al. 2020). 5-FU combined with allicin showed the synergistic antitumour effect on carcinoma cells. Better results were observed when a lower concentration of 5-FU was mixed with allicin than the single-agent treatment at IC50. By preventing migration and colony formation, the co-treatment inhibits tumour cells from spreading and generating secondary sites. This report also established the role of allicin in sensitizing the cancer cells to anticancer drugs (Tigu et al. 2020). Multiple studies have shown that allicin possesses anti-colorectal cancer activity by regulating P38 and STAT pathways and it could prevent or cure colorectal cancer in different stages.

12.7 Resveratrol

Resveratrol (3,5,4′-trihydroxy-trans-stilbene) is a polyphenol belonging to the group of stilbenoids (Farina et al. 2006). It possesses two phenyl rings linked to each other by an ethylene bridge (Fig. 12.7) and exists in two isomeric forms, cis- and trans-resveratrol. The transform is dominant in terms of its prevalence and various biological activities (Salehi et al. 2018). Resveratrol is also a popular phytoalexin and is detected in more than 70 species of plants, which is induced under various biotic and abiotic stress conditions. The various known sources of the compound are grapes, blueberries, raspberries, mulberries, peanuts, cocoa, etc., with 50–100μg/g (0.03–0.14μg/g in grapes and peanuts, respectively) (Sanders et al. 2000; Salehi et al. 2018). It is initially isolated from the roots of white hellebore in 1940, and it gained attention in 1992 for its role in cardioprotective effects of wine (Baur and Sinclair 2006). It is demonstrated in numerous studies that resveratrol possesses a very high antioxidant potential. Further, a number of beneficial health effects, such as anticancer, antiviral, neuroprotective, cardioprotective,

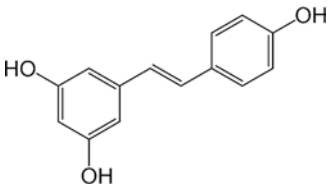


Fig. 12.7 Chemical structure of resveratrol

vasorelaxant, phytoestrogenic, anti-ageing and anti-inflammatory effects, have been reported. Indeed, the anticancer properties have been confirmed by many in vitro and in vivo studies, which show that resveratrol inhibits all the stages of carcinogenesis (Renaud and Ruf 1994; Baur and Sinclair 2006; Salehi et al. 2018).

Resveratrol exhibits anti-inflammatory and anticancer functions in CRC (Honari et al. 2019). Chronic inflammation through the release of cytokines, growth factors and free radicals plays a substantial role in the induction and progression of CRC. Resveratrol suppressed the pro-inflammatory mediators, such as TNF-α and IL-1β, pro-inflammatory enzymes like iNOS and COX-2 and inflammatory signalling pathways such as NF-κB. It also suppresses the free radicals by the upregulation of SOD, catalase (CAT) and glutathione peroxidase (GPX) and activation of the Sirt1/AMPK and Nrf2 pathways (Schaafsma et al. 2016; Honari et al. 2019).

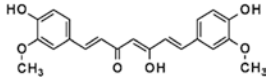
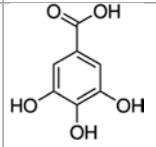
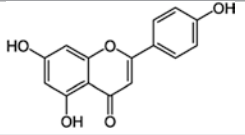
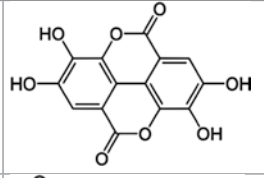
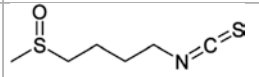
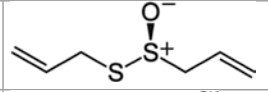
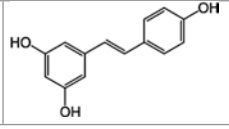
Resveratrol sensitised HT-29 and SW620 cells to 5-fluorouracil, inducing a further increase in oxidative stress, which was linked to the inhibition of AKT and STAT3 proteins (Santandreu et al. 2011). Equally, it strongly modulates the effects of 5-fluorouracil in HCT116 by the suppression of TNF-β-induced activation of tumour-promoting factors (NF-κB, MMP-9, CXCR4) (Buhrmann et al. 2018). Moreover, the viability and invasion of CRC cells were negated by the resveratrol through the suppression of NF-κB-dependent MMP-9 and CXCR4 as well as EMT-related signalling factors including vimentin, slug and E-cadherin (Albini et al. 2011). Resveratrol decreases expression of Wnt target genes, including cyclin D1 and conductin, and suppresses the development of Wnt-induced cells and Wnt-driven CRC cells (Chen et al. 2012a, 2012b). In the in vivo studies on mice and rats, resveratrol could prevent and suppress tumorigenesis in a dose-dependent manner. It induced apoptosis through the increase in expression of both BAX and activated caspase-3 (Schaafsma et al. 2016).

Resveratrol with multiple pleiotropic properties also has a limitation in the form of poor bioavailability. A passive uptake and an active extrusion process are the major challenges. Therefore, many resveratrol derivatives, such as methoxylated, hydroxylated and halogenated derivatives, have been synthesised with all of them exhibiting substantial therapeutic potential (Keylor et al. 2015). Resveratrol and its derivatives could prevent or suppress CRC by modulating Wnt, Akt, Sirt1/AMPK and Nrf2 pathways.

12.8 Conclusion

The use of phytochemicals in cancer treatment/prevention is gaining attention as conventional therapies cause many side effects. Plant foods contain phytochemicals that play a protective role in colorectal cancer prevention by (1) blocking the initiation of carcinogenesis via the induction of detoxifying/antioxidant enzymes and (2) inhibiting the progression of carcinogenesis via the activation of the apoptotic pathway and cell cycle arrest, etc. Table 12.1 describes various phytochemicals that have

Table 12.1 List of bioactive compounds with anti-CRC activity and the pathways they influence in the process

Compound	Source	Structure	Pathways	References
Curcumin	Turmeric		Wnt/β-catenin signalling, PI3K/protein kinase B (Akt), JAK/STAT signalling, MAPK, p53 signalling and NF-κB pathways	Kunnumakkara et al. (2017), Narayan (2004), Johnson et al. (2009), Erkasap et al. (2016), He et al. (2011), Tong et al. (2016)
Gallic acid	Clove buds, strawberries, raspberries, blueberries, black tea, red wine and nuts		Wnt/β-catenin signalling pathway	Lee et al. (2016), Forester et al. (2014), Subramanian et al. (2016)
Apigenin	Fruits, vegetables, onions, oranges, tea, chamomile and wheat sprouts		Intrinsic or extrinsic apoptotic pathway, autophagy, Akt pathway	Banerjee and Mandal (2015), Zhong et al. (2010), Chidambara Murthy et al. (2012), Lee et al. (2016), Dai et al. (2016), Chunhua et al. (2013)
Ellagic acid (EA)	Berries, nuts, grapes, pomegranate, blackberries, strawberries, raspberries, Persian walnut and chestnut		MAPK, PI3K pathways	Girish and Pradhan (2008), Heber (2008), Wada and Ou (2002), Crozier et al. (2006a, 2006b)
Sulforaphane (SFN)	Cauliflower, cabbage, broccoli and other cruciferous vegetables		Caspase and mitochondrial pathway and P38 and JNK pathways and β-catenin pathway	Frydoonfar et al. (2004), Bernkopf et al. (2018)
Allicin	Garlic		P38 and STAT pathways	Bayan et al. (2014), Zhang et al. (2015), Li et al. (2019)
Resveratrol	Grapes, peanuts		Wnt/β-catenin signalling, Sirt1/AMPK and Nrf2 pathways	Chen et al. (2012a, 2012b), Schaafsma et al. (2016), Honari et al. (2019), Albini et al. (2011)

demonstrated anticancer effects using in vitro and in vivo approaches involving these mechanisms.

The molecules discussed in this chapter are reported to target multiple pathways. These phytochemicals are mostly targeting apoptosis through cell cycle arrest. Curcumin, apigenin, ellagic acid, gallic acid, sulforaphane, allicin and resveratrol were shown to regulate apoptosis pathways. However, the process of induction of apoptosis is through different pathways. Gallic acid and apigenin were shown to induce apoptosis by suppressing the activity of cyclin D1 and interfering with cell cycle progression, whereas sulforaphane was reported to hinder the cell cycle through activation of FOXO transcription factor. Other than these, all the molecules were shown to interfere with different signalling pathways that help in disease incidence and progression. Curcumin, gallic acid and sulforaphane molecules showed negative regulation of Wnt/β-catenin pathway, which is one of the aberrant signalling pathways reported in CRC. Along with this, these molecules also target AKT/PI3K-, JAK/STAT-, MAPK- and P53-mediated pathways. Negative regulation of these pathways is shown by all the molecules reported in this chapter. Angiogenesis and metastasis are two important phenomena in disease progression in cancers. Apigenin, ellagic acid, resveratrol and sulforaphane are shown to inhibit these two major events and help in controlling the disease progression. Gallic acid was reported to downregulate cancer stem cells and thus disease progression and drug resistance. Most of these molecules are shown manipulating the inflammatory pathways through the NF-κB pathway and also activating the oxidative stress response enzymes like SOD, catalase and glutathione peroxidase.

Daily exposure to various environmental pollutants, dietary mutagens and carcinogens leads to oxidative stress and inflammatory damages leading to the dysregulation of oncogenes and tumour suppressor genes, aberrant epigenetic alterations and thus the initiation of CRC. The reported dietary phytochemicals in this chapter are comparatively safe with minimum side effects and target multiple cell signalling pathways. They act as chemopreventive molecules by the inhibition of the oxidative stress/inflammation/reactive metabolites of carcinogens by modulating the Nrf2-Keap1 signalling system. The later stages of carcinogenesis are mitigated by induction of apoptosis and cell cycle arrest by influencing the Wnt/β-catenin signalling, PI3K/protein kinase B (Akt), JAK/STAT signalling, MAPK, p53 signalling and NF-κB pathways. There is also a need to further study these phytochemicals for their metabolism, bioavailability, identification of other molecular targets and mechanisms, in vivo studies and investigations of the safe and effective dosages and other studies such as drug-drug interactions and metabolic instability.

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Chapter 13
Seaweeds: Potential Candidates in Human Colon Cancer Therapy

Raghvendra Pandey, Prashant Kumar Singh, and Alok Kumar Shrivastava

Abstract Seaweeds represent some of the most important reservoirs of new remedial compounds for humans. The natural products obtained from seaweeds have received extensive consideration on account of their extraordinary dietary and pharmacology applications, having such antiviral, antifungal, antibacterial, and anticancer properties and so on. Above all, there are a number of natural products that have demonstrated attractive value for the development of novel anticancer agents. This book chapter draws attention on colorectal cancer which is one of the main causes of cancer-related demise of human beings. This chapter also illustrates a range of active natural products extracted from seaweeds that have shown to eliminate or slow the progression of cancer. This also covers the mechanism through which these compounds can induce apoptosis in vitro and in vivo. By considering the ability of compounds present in seaweeds to act against colorectal cancers, this chapter highlights the potential use of seaweeds as anticancer agents.

Keywords Anticancer · Capecitabine · Colon cancer · Marine algae · Seaweeds · 5-FU

13.1 Introduction

Seaweeds or macroalgae are photosynthetic organisms that play a key role in ocean biodiversity and productivity and comprise green algae (Chlorophyta), brown algae (Phaeophyta), and red algae (Rhodophyta). It is the common name for countless

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species of marine plants and algae that grow in the ocean as well as in rivers, lakes, and other water bodies. Some seaweeds are microscopic, such as the phytoplankton that live suspended in the water column and provide the base for most marine food chains. For several centuries, there has been a traditional use of seaweeds as food in China, Japan, and the Republic of Korea. As people from these countries have migrated around the world, this custom has moved with them, so today there are many more countries where the consumption of seaweed is not unusual. Coastal dwellers in tropical countries such as Indonesia and Malaysia have also eaten fresh seaweeds, especially as salad components. There are several seaweeds such as *Porphyra*, *Ulva*, *Laminaria*, *Chlorella*, etc. used as food and its derivatives. There is very little use of seaweeds in various sectors of industries, for instance, food, agriculture, brewery, etc. Besides that, it has several applications in the pharmaceutical industry, since seaweeds contain plentiful biologically active compounds which could provide alternative drugs for several lethal diseases.

From prehistoric times, several marine algae had been used in diets and in traditional medicine, in several countries of the world such as China, Japan, North and South Korea, etc. The increase of algal consumption in Europe and in other parts of the world since the last decades has been witnessed, through the inclusion of new eating habits (Burtin 2003; Mouritsen 2013). Several studies demonstrated a connection between the consumption of marine algae and healthy life expectancy (Jin et al. 2006; Maeda et al. 2005; Nakashima et al. 2009; Lee and Jeon 2013). This may be associated with the truth that marine algae are a reliable source of bioactive compounds which exert a broad range of biological activities, for instance, having antioxidant, antimicrobial, anti-inflammatory, and anticancer properties (Lee et al. 2013; Raja et al. 2013). Moreover, seaweeds are extensively used as functional foods and medicinal herbs and have a long history of use in Asian countries (Namvar et al. 2013). Seaweeds have long been used for the cure of cancer. Many crude or partially purified polysaccharides from various brown, green, and red algae have demonstrated their antitumor properties (Ramberg et al. 2010). A number of studies show that several marine algae constitute a promising resource of novel compounds with potential human therapeutic agents (Pereira et al. 2011).

Cancers are a group of diseases characterized by uncontrolled cell growth and spread (Ferlay et al. 2015). There are several types of cancers reported in human beings, for example, lung, breast, liver, colon, and oral cancers and so on. Among these, colon cancer is the third most common cancer and is considered to be the foremost reason of morbidity and mortality worldwide (Haggar and Boushey 2009). It is also known as colorectal cancer, bowel cancer, or rectal cancer. It has been stated that approximately 55% of colon cancer cases occur in developed regions, on the whole because of lifestyle and eating habits and ecological and environmental conditions of these regions (Haggar and Boushey 2009; Torre et al. 2015). This cancer had been diagnosed firstly in an ancient Egyptian mummy who had lived in the Dakhla Oasis during the Ptolemaic period (Rehemtulla 2010). The majority of colon cancers are because of old age and lifestyle, with only a very few number of cases due to genetic disorders (Bosman Frank 2014). Other risk factors include diet, obesity, smoking, and lack of physical activity (Bosman Frank 2014). Dietary fac-

tors which are responsible for increasing the risk of colon cancer include consumption of red meat, processed meat, and alcohol (Bosman Frank 2014;Theodoratou et al. 2017). About 1.4 million new cases of colon cancer were diagnosed in 2012, which has been reported to be escalating steadily (Kim-Eun et al. 2010; Ferlay et al. 2015). Hungary had the utmost rate of colorectal cancer in 2018, followed by South Korea. The therapy and cure used till date to manage colorectal cancer include a combination of surgery with radiation therapy, chemotherapy (CTX), and targeted therapy, elevating the cost of therapy which the common people are not able to afford. Also, these methods of treatment have a number of side effects; as a result, alternative, low-cost drugs that are easily accessible have to be investigated. It has been noticed that tableware of seafoods and seaweeds represent some of the most vital reservoirs of novel remedial compounds for human beings. Seaweed has been revealed to have quite a lot of biological activities, for instance, anticancer activity (Moussavou et al. 2014).

In this chapter, we have focused on discussing in detail the use of seaweeds in the treatment of human colorectal cancer, and we have summarized the various effects of seaweed-derived compounds on colorectal cancers via promotion of cancer cell apoptosis.

13.2 Secondary Metabolites from Seaweeds

Secondary metabolites are organic compounds produced by bacteria, algae, fungi, and plants which are not directly concerned with the usual growth and development of the organism. The term was first coined by Albrecht Kossel, a Nobel laureate for medicine and physiology in 1910. Thirty years later, a Polish botanist, F. J. F. Czapek, described secondary metabolites as end products of nitrogen metabolism (Bourgaud et al. 2001). Secondary metabolites assist a host in vital functions such as defense, competition, and species interactions, but are not essential for existence. One key defining quality of secondary metabolites is their specificity. Research demonstrated that secondary metabolites can influence different species in different ways. Secondary metabolites are needed for interaction of organisms with their environment and produced as a result of stress. Specific secondary metabolites are generally restricted to a narrow set of species within a phylogenetic group. These compounds generally perform a significant task in plant protection against herbivory and other interspecies defenses. Human beings use these metabolites as medicines, flavorings, pigments, and recreational drugs. The secondary metabolites are found to be the foremost sources of drugs that are natural compounds, which have already demonstrated considerable potential in cancer treatment (Ruiz-Torres et al. 2017; Seca and Pinto 2018a). At least one-third of the existing top 20 drugs are derived from natural sources, including plants and marine species, and among the 175 small molecules approved to treat cancer, 49% are either natural compounds or directly derived from them (Newman and Cragg 2016).

Furthermore, seaweeds are also a source of unique secondary metabolites that showed very interesting bioactivities (El Gamal 2010; Sithranga Boopathy and Kathiresan 2010; Pérez et al. 2016; Wan-Loy and Siew-Moi 2016; Seca and Pinto 2018b).

Due to their high nutritional value, seaweeds have also been used as food in several countries of East Asia (Japan, Korea, and China) and in the Celtic cultures of Europe (Ireland, Scotland, and Brittany) and as an additive in cosmetic and food industries (Rebours et al. 2014; Anis et al. 2017). In the recent past, the chemical profiling of seaweeds unveiled that they are rich in terpenoids, alkaloids, polyphenols, steroids, pigments, and polysaccharides; and some biological assays showed that several of these metabolites have promising pharmacological activities (Gouveia et al. 2013; Cardoso et al. 2015) including in cancer therapy (Folmer et al. 2010; Alves et al. 2018). A number of secondary metabolites have been reported from seaweeds. Among them, the metabolites which are found to be effectual as cure of colon cancer have been discussed below.

13.2.1 The Bioactive Compounds Derived from the Brown Algae and Their Application in Colon Cancer

Bioactive compounds are molecules that can present therapeutic potential with influence on energy intake while reducing pro-inflammatory state, oxidative stress, and metabolic disorders (Siriwardhana et al. 2013). Epidemiological studies indicate that high consumption of foods rich in bioactive compounds with antioxidant activity, including vitamins, phytochemicals, and mainly phenolic compounds, such as flavonoids and carotenoids, has a positive effect on human health and could diminish the risk of numerous diseases, such as cancer, heart disease, stroke, Alzheimer's disease, diabetes, cataracts, and age-related functional decadence (Hassimotto et al. 2009; Siriwardhana et al. 2013).

Bioactive compounds are capable of modulating metabolic processes and demonstrate positive properties such as antioxidant effect, inhibition of receptor activities, inhibition or induction of enzymes, and induction and inhibition of gene expression (Carbonell-Capella et al. 2014). Brown algae contain numerous active compounds; some of them are reported to be curative agents against colon cancer (Table 13.1).

13.2.1.1 Laminarin

Laminarin is a storage glycan composed of β-glycan (β-1,3-β-1,6-glycan) found in brown algae (Fig. 13.1). The effects of laminarin on colorectal cancer cells were investigated in addition to the mechanisms through which laminarin incited apoptosis in these cancerous cells. Treatment with laminarin from *Laminaria* spp. repressed

Table 13.1 Compounds obtained from brown algae and their therapeutic properties against colon cancer

Colon cancer		
Sources (brown algae)	Compounds and their therapeutic properties against cancer	References
Laminaria digitata	Laminarin induced apoptosis in HT-29 colon cancer cells, affected insulin-like growth factor (IGF-IR), decreased mitogen-activated protein kinase (MAPK) and ERK phosphorylation, decreased IGF-IR-dependent proliferation	Park et al. (2012; 2013)
Fucus spp.	Fucoidan has shown activity against both colorectal and breast cancers	Kim-Eun et al. (2010)
Stypopodium sp. *Cystoseira abies-marina*, *Cystoseira usneoides*	Meroditerpenoids are highly effective in colon cancer	Pereira et al. (2011), Gouveia et al. (2013), Amico (1995), Valls and Piovetti (1995)
Undaria pinnatifida	Fucoxanthin attenuated rifampin-induced CYP3A4, MDR1 mRNA, and CYP3A4 protein expression	Yang et al. (2013)

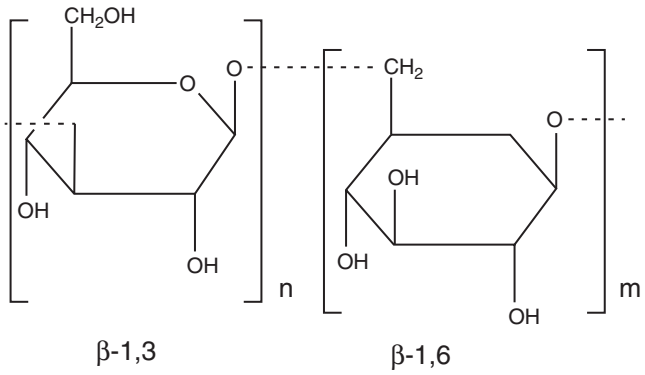

Fig. 13.1 Typical structure of laminarin

the propagation of colon cancer cells via Fas and IGF-IR signaling via the intrinsic apoptotic and ErbB pathways, respectively (Park et al. 2012, 2013). Other studies demonstrated that laminarin-regulated Fas receptors stimulated the activation of members of the caspase family, leading to cleavage of apoptosis markers, for example, ADP-ribose and FADD (Fas-associated protein with death domain) protein levels, suggesting that it stimulated Fas-mediated apoptosis. Laminarin also enhanced the expression of Fas and FADD, which also augmented the activation of caspases (Kischkel et al. 1995; Enari et al. 1996; Salvesen and Dixit 1997).

13.2.1.2 Fucoidan

Fucoidan is a sulfated polysaccharide often found in brown algae. It has shown several biological effects besides anticancer activities (Kim-Eun et al. 2010). Various fucoidan structures (Fig. 13.2) and compositions exist in several brown algal species; nevertheless, in general, the compound consists primarily of L-fucose and sulfate, with little amounts of D-galactose, D-mannose, D-xylose, and uronic acid (Bilan et al. 2002, 2006; Li et al. 2008). Recently, the various biological properties of fucoidan have been described intensively, besides its anticancer activities (Xue et al. 2012). Many studies measured whether fucoidan could hamper the growth of colon cancer cells and studied the molecular pathways involved. A number of authors also stated fucoidan exerts anticancer effects; together with the suppression of growth (Itoh et al. 1993; Zhuang et al. 1995; Riou et al. 1996; Teruya et al. 2007), it also decreased metastasis (Coombe et al. 1987; Ye et al. 2005; Alekseyenko et al. 2007) and inhibited angiogenesis (Ye et al. 2005) in a variety of cancer cells. Fucoidan has been reported to inhibit the growth of a wide range of tumor cells (Alekseyenko et al. 2007; Teruya et al. 2007). It has also been shown to induce apoptosis in colon cancer HT-29 and HCT116 cells in a dose-dependent manner (Kim et al. 2009; Boo et al. 2011). Kim et al. (2009) revealed that low concentrations of fucoidan (5–20 μg/mL) induced apoptosis of HT-29 and HCT116 cells in a dose- and time-dependent manner. However, fucoidan showed a lesser effect on HCT116 cells than on HT-29 cells. According to Hyun et al. (2009)), fucoidan was able to induce apoptosis in HCT15 human colon cancer cells at a concentration of 100 μg/mL. These results showed that the efficacy of fucoidan varied with the type of colon cancer cell studied. It was reported that fucoidan-activated caspases resulted in the induction of apoptosis through both death receptor-mediated and mitochondria-mediated apoptotic pathways (Kim-Eun et al. 2010).

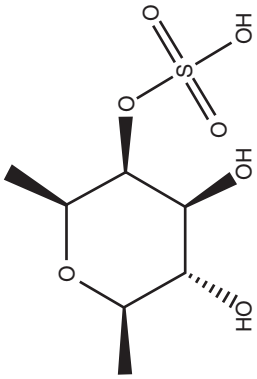

Fig. 13.2 Typical structure of fucoidan

13.2.1.3 Meroterpenoid

Meroterpenoid consists of a terpenoid moiety attached to a toluquinone or a toluhydroquinone ring. *Cystoseira*, a genus of brown algae, have been the source of a collection of this class of meroditerpenoids (Amico 1995; Valls and Piovetti 1995; Gouveia et al. 2013). However, a small number of these compounds have been examined for their biomedical activities, such as antioxidant, antibacterial, and cytotoxic activities. Regarding the antitumor property, the latest reports have elucidated that meroterpenes cystoazorol A and cystoazorones A and B have potential to suppress the growth of HeLa cells (Gouveia et al. 2013; Vizetto-Duarte et al. 2016). Zbakh et al. (2020) demonstrated that the extract of *Cystoseira usneoides* has potential to inhibit the growth of HT-29 colon cancer cells. The extract of *C. usneoides* has antitumor properties due to the presence of several meroterpenoids (Zbakh et al. 2020). The effects and mechanism of action of these algal meroterpenoids (AMTs) on the viability, apoptosis, cell cycle, and motility of HT-29 colon cancer have been demonstrated (Zbakh et al. 2020).

13.2.1.4 Fucoxanthin

Fucoxanthin is a bioactive compound that suppressed the viability of colon cancer cell lines Caco-2, HT-29, and DLD-1 (Caco-2 > DLD-1 > HT-29) and tempted apoptosis in a dose- and time-dependent way, while astaxanthin and β-carotene were not found to alter the viability of Caco-2 cells (Fig. 13.3). The reduced expression intensity of the Bcl-2 protein might contribute to fucoxanthin-induced apoptosis in Caco-2 cells (Hosokawa et al. 2004). Bcl-2 or B-cell lymphoma 2, encoded by the BCL2 gene in humans, is the founding member of the Bcl-2 family of regulator proteins that regulate cell death, by either inhibiting (anti-apoptotic) or inducing (pro-apoptotic) apoptosis (Tsujimoto et al. 1984; Cleary et al. 1986). It was the first apoptosis regulator identified in any organism (Kelly and Strasser 2020).

Kotake-Nara et al. (2005a) assessed the effects of fucoxanthin and neoxanthin on the viability of human colorectal carcinoma Caco-2, human colorectal adenocarcinoma HCT116, mouse melanoma B16, human normal embryonic lung fibroblast MRC-5, and human male umbilical cord fibroblast HUC-Fm cell lines. A study found that fucoxanthin and neoxanthin had nearly the same actions on the cultured cells and were highly efficient in suppressing the viability of HCT116 cancer cells compared to that of the other cancer and normal cell lines. Das et al. (2005) reported

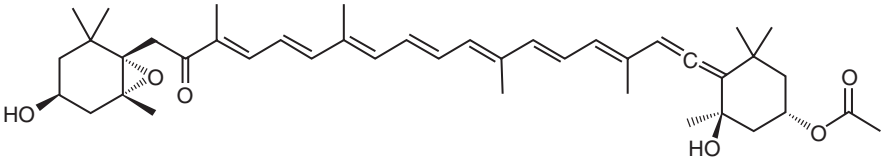

Fig. 13.3 Typical structure of fucoxanthin

that fucoxanthin repressed the proliferation of human colon cancer cell lines WiDr and HCT116 by provoking cell cycle arrest at the G0/G1 phase through upregulating the cyclin-dependent kinase inhibitory protein p21WAF1/Cip1 and retinoblastoma protein (pRb).

Kotake-Nara et al. (2005a) described that fucoxanthin considerably decreased the viability of three human prostate cancer cell lines PC-3, DU145, and LNCaP to 14.9%, 5.0%, and 9.8%, respectively, through apoptosis induction in these cancer cells. The succeeding study stated that fucoxanthin reduced the levels of Bax and Bcl-2 proteins and induced apoptosis in PC-3 cells through caspase-3 activation (Kotake-Nara et al. 2005a, 2005b). Yoshiko and Hoyoko (2007) showed that fucoxanthin induced cell cycle arrest at the G1 phase and GADD45A expression and inhibited the growth of DU145 cells. In their study, Satomi and Nishino (2009)) showed that several MAPKs modulated the induction of GADD45 and G1 arrest and positive regulation by SAPK/JNK was involved in GADD45A induction and G1 arrest by fucoxanthin, indicating that GADD45A was closely related with the G1 arrest induced by fucoxanthin, and MAPK pathways were implicated in fucoxanthin-induced GADD45A expression and G1 cell cycle arrest in tumor cells depending on the cell type.

13.2.2 The Bioactive Compounds Isolated from the Green Algae and Their Application in Colon Cancer

Several investigations have concentrated on the biological actions of plant-derived phytochemicals, but very few were about the phytochemicals derived from microalgae (Kwang et al. 2008). Microalgae-derived phytochemicals have more potential biological activities than the those of terrestrial origin (plant phytochemicals) (Holst and Williamson 2008; Prabakarana et al. 2018). Microalgae have an assortment of phenolic classes that are quite different from many other plant species (medicinal plants, fruits, and vegetables). Chlorophyll and carotenoid content is higher in microalgae than in some plants (Villarruel López et al. 2017).

The effects of microalgae's anticancer activity are complex because of their significant structural diversity, which entails multiple interactions. Microalgae are a great source of several useful by-products like carbon compounds; these carbon compounds have medical, cosmetic, and pharmaceutical uses (Das et al. 2011). Moreover, several constituents are present in microalgae such as lipids, proteins, polysaccharides, vitamins, and antioxidants (Brennan and Owende 2010). Microalgae enhance the host's defense by increasing natural killer cell activity (Yuan and Walsh 2006), activation of the immune system (Schumacher et al. 2011), and inhibition of cancer cell growth (Liu et al. 2012). Thus, microalgae were hypothesized as a contributing factor in the inhibition of carcinogenesis. Green algae contain numerous active compounds; some of them are reported to be curative agents against colon cancer. In this part of the book chapter, we have attempted to discuss the gainful part of green microalgal bio-products that fundamentally can be utilized as anticancer agents (Table 13.2).

Table 13.2 Compounds obtained from green algae and their therapeutic properties against colon cancer

Sources (green algae)	Compounds and their therapeutic properties against cancer	References
Chlorella ellipsoidea	Carotenoids enhancing the fluorescence intensity of the early apoptotic cell population in HCT116 cells	Abd El-Hack et al. (2018)
Cymopolia barbata	Prenylated bromohydroquinones (PBQs) show selectivity and potency against HT-29 cells and inhibit CYP1 enzyme activity, which may lead to chemoprevention	Badal et al. (2012)
Ulva fasciata	Flavonoids are highly effective against colon cancer	Ruy et al. (2013)
Enteromorpha intestinalis	Methanols are highly effective against colon cancer	Paul et al. (2013)

The antiproliferative activity of carotenoids separated from marine *Chlorella ellipsoidea* has been evaluated. HPLC analysis revealed that the main carotenoid from *C. ellipsoidea* was composed of violaxanthin with two minor xanthophylls, antheraxanthin and zeaxanthin (Cha et al. 2008). In addition, treatment with both *Chlorella* extracts enhanced the fluorescence intensity of the early apoptotic cell population in HCT116 cells. The *C. ellipsoidea* extract produced an apoptosis-inducing effect almost 2.5 times stronger than that of the *C. vulgaris* extract. These results indicate that bioactive xanthophylls of *C. ellipsoidea* might be useful functional ingredients in the prevention of human cancers.

The extract of *Cymopolia barbata* (green marine algae) has significant pharmacological properties such as antifungal, antitumor, antimicrobial, and antimutagenic activities. Even if the cymopols are known halogenated natural products which have been isolated from *C. barbata*, active ingredients responsible for the displayed biological activities remain unspecified. Badal et al. (2012) demonstrated bioactivities of prenylated bromohydroquinones (PBQs), cymopol-related metabolites obtained from *Cymopolia barbata*. Compounds 7-hydroxycymopochromanone (PBQ1) and 7-hydroxycymopolone (PBQ2) (Fig. 13.4) isolated from *Cymopolia barbata* were examined for cytotoxicity against three cancerous cell lines besides their potential for chemoprevention by means of inhibition of cytochrome P450 (CYP) 1 enzymes. The CYP1 family of enzymes, especially CYP1B1, appears to be a universal molecular marker and a target for drug discovery against cancer. Several natural products have been found to be direct inhibitors of CYP1 enzymes and generate metabolites that are CYP inhibitors with cytotoxic properties. Badal et al. (2012) demonstrated PBQ2 is a potent inhibitor of CYP1B1 activity, together with promising and specific activity against the colon cancer cell line HT-29.

The *Ulva fasciata* extract (UFE) from *Ulva fasciata* Delile (sea lettuce), which grows abundantly along coastal seashores, was used to assess the mechanisms underlying the cytotoxicity of green algae (Ryu et al. 2013). The antiproliferative effects of the *Ulva fasciata* extract against colon cancer cells involved the induction of apoptosis. Reactive oxygen species have been reported to control apoptotic signal transduction and persuade depolarization of the mitochondrial membrane, leading to increased levels of pro-apoptotic molecules in the cytosol (Lin 1999; Li et al. 2000). Li et al., (2000) demonstrated that the *Ulva fasciata* extract significantly

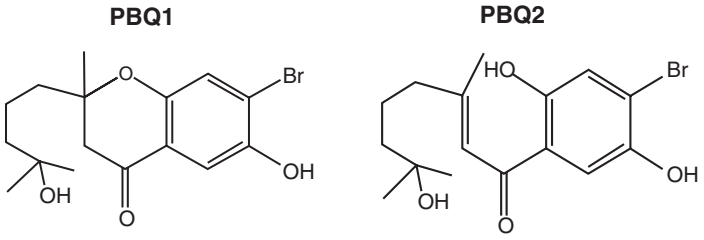

Fig. 13.4 Typical structure of 7-hydroxycymopochromanone (PBQ1) and 7-hydroxycymopolone (PBQ2)

increased reactive oxygen species generation in HCT116 cells and that antioxidant-mediated scavenging of UFE-induced reactive oxygen species reduced the UFE-mediated cell death. UFE was able to inhibit the growth of HCT116 human colon cancer cells by 50% at a concentration of 200 μg/mL. It induced apoptosis through alteration in Bcl-2 family protein expression, increasing mitochondrial membrane permeability, and activation of caspase 9 and caspase 3 (Ryu et al. 2013). Accumulating evidence suggests that bioactive compounds extracted from algae produce anticancer effects through multiple mechanisms of action, including inhibition of cancer cell growth, invasion, and metastasis, and through the induction of apoptosis in cancer cells (Farooqi et al. 2012).

Enteromorpha intestinalis are members of green macroalgae known as Chlorophyceae. These macroalgae have already been studied for antioxidant and antimicrobial activities. Al-Jaber et al. (2015) demonstrated cytotoxic activity of the *Enteromorpha intestinalis* extract on HeLa cells. GC/MS analysis revealed that the extract contains loliolide, palmitic acid, ethyl palmitate, phytol, and squalene. The acetone extract of *E. intestinalis* was revealed to be highly effective on human colon carcinoma LS174 cells (Kosanić et al. 2014).

13.2.3 The Bioactive Compounds from the Red Algae and Their Application in Colon Cancer

Marine algae accumulate high levels of minerals from seawater over their lifetime (Aslam et al. 2009). The proliferation and differentiation of human colon carcinoma cell lines were assessed in the presence of a mineral-rich extract from the red marine alga *Lithothamnion calcareum* (Aslam et al. 2009). The algal extract was as effective as inorganic calcium in both inhibition of colon carcinoma cell growth and induction of its differentiation. Both epidemiological studies (Bostick et al. 1993; Kampman et al. 1994, 2000; McCullough et al. 2003; Flood et al. 2005) and interventional studies (Baron et al. 1999; Grau 2003) in humans have demonstrated that calcium has the capacity to reduce polyp formation in the colon. Other studies have found that different minerals obtained from marine algae could also contribute to the reduction of polyp formation. In another study, Aslam et al. (2009) reported that

Table 13.3 Compounds obtained from red algae and their therapeutic properties against colon cancer

Sources (red algae)	Compounds and their therapeutic properties against cancer	References
Lithothamnion calcareum or *Phymatolithon calcareum* (Pallas)	Multi-mineral product that can protect against adenomatous polyp formation in the colon	Aslam et al. (2009)
Laurencia spp.	Effects of dactylone have been studied in many cancer cell lines, including human colon cancer HCT116 cells	Fedorov et al. (2007)
Hypnea saidana	Epoxydon *(Phoma herbarum)* plays an important role in colon cancer	
Porphyra haitanensis	*Porphyra haitanensis* polysaccharides (PHPs) have a crucial role in colon cancer therapy	Yao et al. (2020)
Lophocladia sp.	Lophocladines have an important role in colon cancer therapy	
Plocamium telfairiae	Methanolic extract is effective against colon cancer	Kim-Eun et al. (2007)

a multi-mineral product obtained from marine algae was able to reduce colon polyp formation in C57BL/6 mice receiving either a high-fat diet or a low-fat diet. Similarly to brown and green algae, red algae also contain numerous active compounds; some of them are reported to be therapeutic agents against colon cancer listed in Table 13.3.

13.2.3.1 Extract of *Lithothamnion calcareum*

An earlier study suggested that dietary aquamin, a calcium-rich, multi-mineral natural product, checked colon polyp formation and transition to invasive tumors more effectively. McClintock et al. (2018) suggested that combining calcium with additional trace elements may provide a way to enhance the beneficial effects of calcium in the colon. This natural product (aquamin), consisting of the skeletal remains of red marine algae of the *Lithothamnion* family (McClintock et al. 2018) and containing magnesium as well as detectable amounts of 72 additional trace elements in addition to calcium, suppressed colon polyp formation and transition to invasive tumors in C57BL/6 mice on a high-fat diet more effectively (Aslam et al. 2010, 2012). In studies with human colon carcinoma cells in monolayer culture, aquamin was more effective than calcium alone at suppressing tumor cell growth and inducing differentiation (Aslam et al. 2009; Singh et al. 2015). The studies demonstrated that calcium does, in fact, affect growth in human colon adenomas obtained from large tumor specimens in colonoid culture. The studies showed, furthermore, that a multi-mineral approach has the capacity to modulate structure and function in these specimens at calcium concentration that is ineffective with calcium alone. At the same time, there is no evidence of toxicity for normal colonic mucosa in colonoid culture (McClintock et al. 2018).

13.2.3.2 Dactylone

Dactylone is representative of a new group of natural cancer-preventive agents (Fedorov et al. 2007). Structurally, it is closely related to the sesquiterpenoids extracted from red algae *Laurencia* spp. (Fig. 13.5). The effects of dactylone have been studied in several cancer cell lines, apart from human colon cancer HCT116 cells, and the molecular mechanism underlying these effects was measured (Fedorov et al. 2007). Dactylone was able to inhibit the phenotype expression of several human cancer cell lines and was revealed to persuade G1-S cell cycle arrest and apoptosis in tumor cells; it reduced phosphorylation of the Rb protein at Ser795, Ser780, and Ser807/811 sites and also repressed the expression of cyclin D3 and cyclin-dependent kinase (CDK) 4 (Fedorov et al. 2007).

13.2.3.3 *Porphyra haitanensis* Polysaccharides (PHPs)

Anticancer effects of *Porphyra haitanensis* polysaccharides (PHPs) on human colon cancer cells and noncancerous cells were evaluated. Cytotoxicity test showed that PHPs had inhibitory effects on the growth of colon cancer cells HT-29, LoVo, and SW-480, but no toxic effects on the normal human cells HaCaT. Studies suggested that polysaccharides from *P. haitanensis* have anticancer effects on human colon cancer cells and therefore might be regarded as new candidates for the prevention and treatment of colon cancers (Yao et al. 2020).

13.2.3.4 Lophocladines

Lophocladines A (**1**) and B (**2**) (Fig. 13.6), two 2,7-naphthyridine alkaloids, were isolated from the marine red alga *Lophocladia* sp. collected in the Fijian Islands. Their structures were deduced on the basis of high-resolution mass spectrometry and one- and two-dimensional NMR spectroscopy. Lophocladine A (**1**) displayed affinity for NMDA receptors and was found to be a δ-opioid receptor antagonist, whereas lophocladine B (**2**) exhibited cytotoxicity to NCI-H460 human lung tumor and MDA-MB-435 breast cancer cell lines. Immunofluorescence studies indicated that the cytotoxicity of lophocladine B (**2**) correlated with microtubule inhibition. This is the first reported occurrence of alkaloids based on a 2,7-naphthyridine skeleton from red algae.

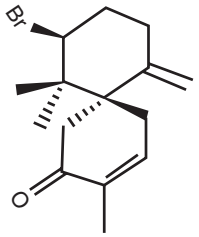

Fig. 13.5 Typical structure of dactylone

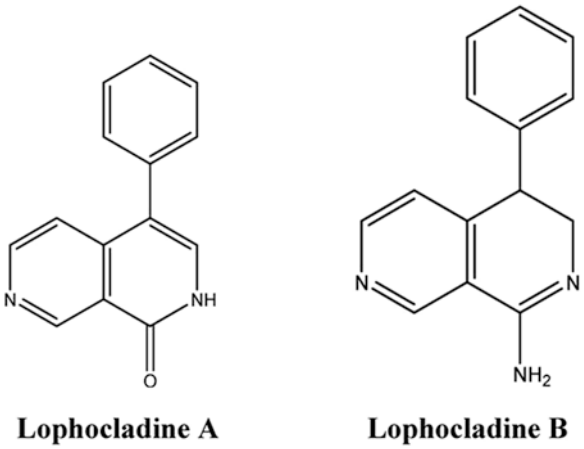

Fig. 13.6 Typical structure of lophocladine A and lophocladine B

13.2.3.5 Methanolic Extract of *Plocamium telfairiae*

Methanolic extracts from *Plocamium telfairiae* (PTE) revealed a cytotoxic effect against colon carcinoma cells (HT-29) of human beings. Kim et al. (2007) demonstrated the cytotoxic activity and mechanism of PTE-induced apoptosis in human colon cancer cells (in HT-29 cells). Application of PTE on HT-29 resulted in the inhibition of growth and induction of apoptosis in a dose-dependent manner. PTE have potential to function as a chemopreventive as well as chemotherapeutic agent in HT-29 cells of human beings by means of the diminution of cell viability and the stimulation of apoptosis (Kim et al. 2007).

13.2.4 Structure and Pharmacokinetics of the Chemotherapeutic Drugs Commonly Used for Colon Cancer

Pharmacokinetics is a branch of pharmacology dedicated to determine the fate of substances administered to a living organism. The substances of interest include any chemical xenobiotic such as pharmaceutical drugs, pesticides, food additives, cosmetics, etc. It attempts to analyze chemical metabolism and to discover the fate of a chemical from the moment that it is administered up to the point at which it is completely eliminated from the body. Pharmacokinetics is the study of how an organism affects a drug, whereas pharmacodynamics (PD) is the study of how the drug affects the organism. Both influence dosing, benefit, and adverse effects, as seen in PK/PD models.

Pharmacokinetics describes how the body affects a specific xenobiotic/chemical after administration through the mechanisms of absorption and distribution, as well as the metabolic changes of the substance in the body and the effects and routes of excretion of the metabolites of the drug. Pharmacokinetic properties of chemicals

are affected by the route of administration and the dose of the administered drug. These may affect the absorption rate (Bryant et al. 2018).

Chemotherapy (CTX) is a type of cancer treatment that uses one or more anticancer drugs as part of a standardized chemotherapy regimen. Chemotherapy may be given with a curative intent (which almost always involves combinations of drugs), or it may aim to prolong life or to reduce symptoms (palliative chemotherapy). Chemotherapy is one of the major categories of the medical discipline specifically devoted to pharmacotherapy for cancer, which is called medical oncology (Alfarouk et al. 2015; Johnstone et al. 2002). These therapies with specific molecular or genetic targets, which inhibit growth-promoting signals from classic endocrine hormones (primarily estrogens for breast cancer and androgens for prostate cancer) are now called hormonal therapies. By contrast, other inhibitions of growth signals like those associated with receptor tyrosine kinases are referred to as targeted therapy.

Importantly, the use of drugs (whether chemotherapy, hormonal therapy, or targeted therapy) constitutes systemic therapy for cancer in that they are introduced into the bloodstream and are therefore in principle able to address cancer at any anatomic location in the body. Systemic therapy is often used in conjunction with other modalities that constitute local therapy (i.e., treatments whose efficacy is confined to the anatomic area where they are applied) for cancer such as radiation therapy, surgery, or hyperthermia therapy.

Traditional chemotherapeutic agents are cytotoxic by means of interfering with cell division, but cancer cells vary widely in their susceptibility to these agents. To a large extent, chemotherapy can be thought of as a way to damage or stress cells, which may then lead to cell death if apoptosis is initiated. Many of the side effects of chemotherapy can be traced to damage to normal cells that divide rapidly and are thus sensitive to antimitotic drugs: cells in the bone marrow, digestive tract, and hair follicles. This results in the most common side effects of chemotherapy: myelosuppression (decreased production of blood cells, hence also immunosuppression), mucositis (inflammation of the lining of the digestive tract), and alopecia (hair loss). Because of the effect on immune cells (especially lymphocytes), chemotherapy drugs often find use in a host of diseases that result from harmful overactivity of the immune system against self (so-called autoimmunity). These include rheumatoid arthritis, systemic lupus erythematosus, multiple sclerosis, vasculitis, and many others. Some drugs commonly used for colon cancer are irinotecan (Camptosar), oxaliplatin (Eloxatin), capecitabine (Xeloda), 5-fluorouracil (5-FU), and trifluridine (TFD) and tipiracil (TPI) (Lonsurf).

13.2.4.1 Structure and Pharmacokinetics of Irinotecan

Irinotecan is an antineoplastic enzyme inhibitor mainly employed in the management of colon cancer (Fig. 13.7). It is derived from camptothecin that hampers the activity of enzyme topoisomerase I. It stops religation of the DNA strand and causes double-strand DNA breakage and thus cell death. Irinotecan (trade name Onivyde) was permitted for the treatment of advanced pancreatic cancer. Irinotecan demon-

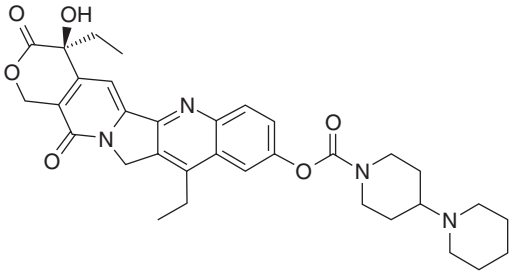

Fig. 13.7 Typical structure of irinotecan

strates better water solubility as compared to camptothecin. Irinotecan is a yellow crystalline powder, soluble in water and glacial acetic acid, partially soluble in chloroform, and slightly soluble in methanol (Chabot 1997).

Early in phase I studies, irinotecan displayed some activity in various diseases including colorectal, lung, and cervical cancers (Negoro et al. 1991; Ohe et al. 1992; Rothenberg et al. 1993; Catimel et al. 1995). Most investigators noted activity at the higher dosages administered, which is indicative of a dose-response relationship with this drug. In phase II studies, irinotecan has demonstrated antitumor activity in several types of cancer including colorectal, lung, cervical, pancreatic, stomach, and breast cancers (Fukuoka et al. 1992; Masuda et al. 1992; Shimada et al. 1993; Armand et al. 1995; Boisseau et al. 1995; Rothenberg 1996; Conti et al. 1996; Irvin et al. 1998). Its activity has also been documented in leukemias and lymphomas.

Irinotecan is extensively metabolized in the liver of a human being (Rivory et al. 1996). The key dose-limiting toxicities of this drug were noticed to be diarrhea and neutropenia. Other undesirable effects included nausea, vomiting, and alopecia along with an acute cholinergic syndrome during drug administration (Gandia et al. 1993).

13.2.4.2 Structure and Pharmacokinetics of Oxaliplatin

Oxaliplatin was discovered in 1976 at Nagoya City University by Professor Yoshinori Kidani. Oxaliplatin was consequently in-licensed by Debiopharm and developed as an advanced colon cancer treatment (Fig. 13.8). Oxaliplatin is used for treatment of colon cancer, typically along with folinic acid and 5-fluorouracil in a combination known as FOLFOX. Oxaliplatin has been compared with other platinum compounds used for advanced cancers, such as cisplatin and carboplatin. Oxaliplatin, sold under the brand name Eloxatin, is a cancer medication used to treat colon cancer. Often it is used together with fluorouracil and folinic acid (leucovorin) in advanced cancer. It is given by injection into a vein ("Oxaliplatin", The American Society of Health-System Pharmacists 2016).

Common side effects include numbness, feeling tired, nausea, diarrhea, and low blood cell counts (Oun et al. 2018). Other serious side effects include allergic reactions (Oun et al. 2018). Use in pregnancy is known to harm the baby. Oxaliplatin is in the platinum-based antineoplastic family of medications (Apps et al. 2015). It is assumed to work by blocking the DNA replication.

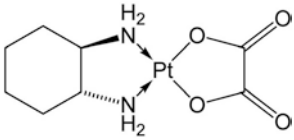

Fig. 13.8 Typical structure of oxaliplatin

In contrast to cisplatin and carboplatin, oxaliplatin features the bidentate 1, 2-diaminocyclohexane in place of the two monodentate ammine ligands. It also features a bidentate oxalate group (Apps et al. 2015). The 3D structure of oxaliplatin has been revealed by X-ray crystallography, though the occurrence of pseudo-symmetry in the structure has caused uncertainty in its interpretation (Johnstone 2014).

According to in vivo studies, oxaliplatin fights carcinoma of the colon through non-targeted cytotoxic effects. Like other platinum compounds, its cytotoxicity is thought to result from inhibition of DNA synthesis in cells. In particular, oxaliplatin forms both inter- and intra-strand cross-links in DNA (Graham et al. 2004), which prevent DNA replication and transcription, causing cell death.

Oxaliplatin by itself has unassuming action against advanced colon cancer (Bécouarn et al. 1998). When compared with just 5-fluorouracil and folinic acid administered according to the de Gramont regimen, a FOLFOX4 regime produced no significant increase in overall survival, but did produce an improvement in progression-free survival, the primary end point of the phase III randomized trial (De Gramont et al. 2000).

Side Effects of Oxaliplatin

- Neurotoxicity leading to chemotherapy-induced peripheral neuropathy; a progressive, enduring, and often irreversible tingling numbness; intense pain; and hypersensitivity to cold, beginning in the hands and feet and sometimes involving the arms and legs, often with deficits in proprioception (Pasetto et al. 2006). This chronic neuropathy may also be preceded by a transient acute neuropathy occurring at the time of infusion and associated with excitation of voltage-gated Na^+ channels (Webster et al. 2005; Chay et al. 2010).
- Fatigue.
- Nausea, vomiting, or diarrhea.
- Decrease in the number of neutrophil cells (neutropenia).
- Loss of hearing (ototoxicity).
- Leakage of oxaliplatin from the infusion vein causes severe damage to the connective tissues (extravasation).
- Lower blood potassium (hypokalemia), which is more common in women than men (Chay et al. 2010).
- Persistent hiccups.
- Rhabdomyolysis.
- Additionally, a number of patients may experience an allergic reaction to platinum-containing drugs which is very common in the case of women (Chay et al. 2010).

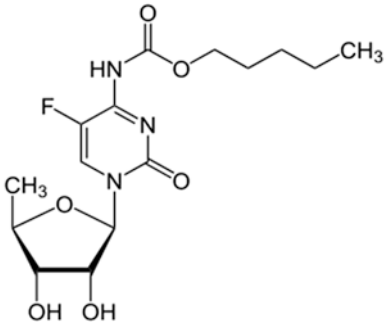

Fig. 13.9 Typical structure of capecitabine

13.2.4.3 Structure and Pharmacokinetics of Capecitabine

Capecitabine is a carbamate ester that is cytidine in which the hydrogen at position 5 is replaced by fluorine and in which the amino group attached to position 4 is converted to its N-(penyloxy)carbonyl derivative (Fig. 13.9). Capecitabine is an antineoplastic drug employed in the cure of cancers. Capecitabine is an oral prodrug of 5-fluorouracil and is often employed for the management of breast, colorectal, as well as gastric cancer. After oral administration, capecitabine is quickly and fully absorbed. Afterward, capecitabine is metabolized to subsequently 5′-deoxy-5-fluorocytidine, 5′-deoxy-5-fluorouridine, and 5-fluorouracil passing through a three-step enzymatic cascade involving the enzymes carboxylesterase, cytidine deaminase, and thymidine phosphorylase, respectively (Judson et al. 1999; Reigner et al. 2001). Around 80% of 5-fluorouracil is rapidly catabolized to inactive metabolites, and a small proportion of 5-fluorouracil is intracellularly anabolized to cytotoxic metabolites (Diasio and Harris 1989; Longley et al. 2003). The enzyme dihydropyrimidine dehydrogenase catalyzes the initial step of 5-fluorouracil catabolism that leads to the formation of 5,6-dihydro-5-fluorouracil. 5,6-Dihydro-5-fluorouracil is eventually metabolized to fluoro-β-alanine, which is cleared renally (Heggie et al. 1987; Reigner et al. 2001). The capecitabine absorption rate was highly variable between subjects and occasions.

13.2.4.4 Structure and Pharmacokinetics of 5-Fluorouracil

5-Fluorouracil (5-FU), sold under the brand name Adrucil, is a medication employed to cure cancer (Fig. 13.10). It is used by injection for treatment of various types of cancer such as colon cancer, esophageal cancer, stomach cancer, pancreatic cancer, breast cancer, and cervical cancer. As a cream, it is used against actinic keratosis, basal cell carcinoma, and skin warts.

When this is used in the form of injection, most people develop side effects. Common side effects include inflammation of the mouth, loss of appetite, low blood cell counts, hair loss, and inflammation of the skin. When used as a cream, irritation at the site of application usually occurs (*The American Society of Health-System*

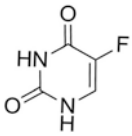

Fig. 13.10 Typical structure of 5-fluorouracil

Pharmacists, 2016). Use of either form in pregnancy may harm the baby. Fluorouracil is in the antimetabolite and pyrimidine analog families of medications (Airley 2009). How it works is not entirely clear but believed to involve blocking the action of thymidylate synthase and thus stopping the production of DNA. Fluorouracil was patented in 1956 and came into medical use in 1962.

The current chemotherapeutic agents used in the treatment of colon cancer, namely, 5-fluorouracil, are associated with adverse effects and development of chemoresistance, a major clinical limitation (He et al. 2017). 5-Fluorouracil treatment ultimately fails in over 90% of patients with metastatic cancer, and many mechanisms involved in drug resistance such as evasion of apoptosis were attributed to this finding (Longley et al. 2006; Hu et al. 2016).

One of the strategies to overcome the main limitations associated with the use of anticancer agents, namely, 5-fluorouracil, is the combination therapy that consists of simultaneous administration of a natural compound and an anticancer agent. Natural compounds appear as interesting adjuvants attributed to low toxicity in normal cells and the ability to target multiple pathways, thus reducing development of drug resistance and enhancing the activity of anticancer agents (Redondo-Blanco et al. 2017).

13.2.4.5 Structure and Pharmacokinetics of Trifluridine and Tipiracil (Lonsurf)

Since the synthesis of 5-fluorouracil in 1957 (Hoff et al. 2001), fluoropyrimidines have been used to treat several types of cancer (Hong et al. 2006). Due to the drawbacks of 5-FU therapy, for example, having to be administered over long periods of time via intravenous infusion and the development of resistance in tumors, more convenient and efficacious fluoropyrimidine therapy has been desired (Hong et al. 2006). The fluoropyrimidine component of this drug, trifluridine, was first synthesized in 1964 by Heidelberger et al. (Hong et al. 2006). Trifluridine/tipiracil (TFD/TPI) (Lonsurf) is pharmaceutical product that is used as a third- or fourth-line treatment of metastatic colon cancer, after chemotherapy and targeted therapeutics have failed (Fig. 13.11). It is a mixture of two active pharmaceutical components: trifluridine, a nucleoside analog, and tipiracil, a thymidine phosphorylase inhibitor. Tipiracil prevents trifluridine from being metabolized quickly, thus increasing the bioavailability of trifluridine (Hoff et al. 2001; Kish and Uppal 2016).

TFD primarily binds to human serum albumin. The in vitro protein binding of TFD in human plasma is greater than 96%, independent of the drug concentration and the presence of TPI. The plasma protein binding of TPI is less than 8%. Neither

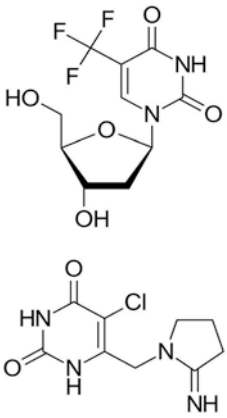

Fig. 13.11 Typical structure of trifluridine and tipiracil

TFD nor TPI is metabolized by cytochrome P450 enzymes. TFD is mainly eliminated by metabolism via thymidine phosphorylase to form an inactive metabolite, 5-(trifluoromethyl)uracil. No other major metabolites were detected in plasma or urine (Kish and Uppal 2016). The combination caused harm to the fetus of pregnant animals, and it was not tested in pregnant women. Pregnant women should not take it, and women should not become pregnant while taking it.

13.2.4.6 Adverse Effects

The combination severely suppresses bone marrow function, resulting in fewer red blood cells, white blood cells, and platelets, so many people taking it are at risk of infections, anemia, and blood loss from lack of clotting. It also causes digestive problems, with more than 10% of people experiencing loss of appetite, diarrhea, nausea, and vomiting. More than 10% of people experience fatigue and fever (Hong et al. 2006).

13.2.5 Commercialization of the Seaweed-Derived Drug for Colon Cancer and Brand

Due to the rising cases of cancer in both developing and developed countries of the worlds, the application of novel chemotherapeutic molecules is required (Pereira et al. 2011). Using natural or synthetic molecules to prevent or repress the progress of invasive cancers has recently been identified as an approach with enormous potential (Mann et al. 2005).

Seaweeds are comprehensively used as functional foods and medicinal herbs and have a long history of use in Asian countries (Namvar et al. 2013). As definite seaweeds have long been employed for the cure of cancer, a lot of crude or partially

purified polysaccharides from various brown, green, and red algae have been tested for their antitumor properties (Ramberg et al. 2010). Several studies have shown that marine algae form a promising source of novel compounds with potential as human therapeutic agents. In particular, algae have been considered as a potential source of new bioactive compounds (Pereira et al. 2011). Many studies have reported that compounds extracted from seaweeds may be effective anticancer agents. Several investigations have aimed to find effective ways to combat colorectal cancer. A few studies have reported that colorectal cancer can be successfully treated with marine natural products, which contain an abundance of biologically active substances with novel chemical structures and favorable pharmacological activities (Ryu et al. 2013).

The prevention of apoptosis in colorectal cancer cells increases tumor growth, supports neoplastic development, and bestows resistance to cytotoxic anticancer agents (Bedi et al. 1995). Therefore, bioactive compounds that persuade apoptosis in cancer cells can be used as agents for cancer chemoprevention and/or chemotherapy (Kim-Eun et al. 2010). Accumulating facts advocate that bioactive compounds extorted from algae produce anticancer effects through a variety of mechanisms of action, including suppression of cancer cell growth, invasion, and metastasis, and through the stimulation of apoptosis in cancer cells (Farooqi et al. 2012) (Table 13.4). Apoptosis may be commenced either by a mitochondria-mediated (intrinsic) or a death receptor-mediated (extrinsic) pathway (Brenner and Mak 2009; Mellier et al. 2010). Each of these pathways involves the activation of caspases and ultimately leads to apoptosis (Park et al. 2012).

Meroditerpenoids such as plastoquinones, chromanols, and chromenes are a class of natural products containing a polyprenyl chain attached to a hydroquinone ring moiety and are generally found in brown algae (Pereira et al. 2011). Epitaondiol,

Table 13.4 List of seaweed-derived compounds and their target sites

			Action site			References	
Therapeutic compounds (seaweed)	Cell cycle arrest	Mitochondrial membrane	Caspases or cyclins	GFR	P53	Pro- or anti-apoptotic proteins	
Fucoidan (*Fucus* spp.)	+	+	+	+		+	Kim-Eun et al. (2010), Xue et al. (2012)
Laminarin (*Laminaria* spp.)	+	+	+	+		+	Park et al. (2012; 2013)
Dactylone (*Laurencia* spp.)	+	–	+	+			Fedorov et al. (2007)
Sterol fraction (*Porphyra dentata*)	+	–	–	–	–	_	Kazlowska et al. (2013)
Methanol extracts (*Sargassum muticum)*	+	–	–	–	–	_	Paul et al. (2013)

+ effects reported; – no effects reported

epitaondiol diacetate, epitaondiol monoacetate, stypotriol triacetate, 14-ketostypodiol diacetate, and stypodiol extracted from *Stypopodium flabelliforme* inhibited cell proliferation in five cell lines: human neuroblastoma (SH-SY5Y), rat basophilic leukemia (RBL-2H3), murine macrophage (Raw267), Chinese hamster fibroblast (V79), and human colon adenocarcinoma (Caco-2) cells (Pereira et al. 2011). Stypotriol triacetate demonstrated the main prevention of the colon adenocarcinoma cell line Caco-2, followed by epitaondiol monoacetate and epitaondiol. Several studies have investigated the application of seaweed in the fight against many diseases, including colorectal cancers. Laminarin tempted apoptosis via the Fas and IGF-IR signaling pathways and via the intrinsic apoptotic and ErbB pathways (Jeong and Seol 2008).

Fucoidan-containing seaweeds have been well recognized and applied for their anticancer properties for centuries. This includes being traditionally used in supplements and drinks administered to cancer patients in Korea, Japan, China, and other countries. In nature, fucoidan plays a crucial task in defending seaweeds from pathogens and environmental damage. Research demonstrates that fucoidan also expands its defensive property into a wide range of human health areas, including integrative oncology. Current research continues to add to the growing body of evidence that fucoidan is a secure and efficient component in complementary cancer therapies.

Australian company Marinova is leading the way in fucoidan science. The company specializes in the research and manufacture of highly bioactive fucoidan extracts derived from the *Fucus vesiculosus* and *Undaria pinnatifida* seaweed species. Marketed under the Maritech brand, these unique ingredients are the only high-purity, certified-organic fucoidans in the world and are highly sought after for use in nutritional, dietary, and pharmaceutical applications. Fucoidan is understood to impart anticancer activities through various pathways. Maritech fucoidan has been shown in preclinical research to cause cell cycle arrest in the first growth phase (G1) of a HCT116 human colon cancer cell line.

Healthy tissues and cells can also be affected by the toxicity of the treatment, leading to problematic side effects. Fucoidan has been well studied for its ability to protect against chemotherapy toxicity. It shows significant potential in alleviating a number of common side effects caused by cancer treatment.

The chemical nature, structure, and bioactivity of fucoidan are basically based upon the species of seaweed from which it is isolated and its method of extraction. Maritech fucoidan is isolated from wild grown *Fucus vesiculosus* and *Undaria pinnatifida* seaweeds sourced from the pristine coastal waters of Tasmania, Patagonia, Nova Scotia, and Brittany.

13.3 Conclusion

Colon cancer is the third most common cancer in men and second most common cancer in women and is widespread throughout the world (Alwarsamy et al. 2016; Siegel et al. 2014). Globally, the occurrence of this disease is rising, apparently

owing to changes in food habits. Food habits can influence the growth and development of colon cancer in human beings, and food ingredients could act as chemotherapeutic agents (Kim et al. 2003; Pollak et al. 2004; Hyun et al. 2009; Kim-Eun et al. 2010; Thinh et al. 2013; Han et al. 2015b; Somasundaram et al. 2016; Han et al. 2017). Many researchers have explored possible treatments for colon cancer. A number of studies have advocated marine natural products with pharmacological properties as a method for treating colon cancer (Fedorov et al. 2007). Among these marine natural products, seaweeds have been well documented to have a range of valuable compounds such as laminarin, fucoidan, dactylone, and meroditerpenoids with diverse effects on cancerous cells (Kim et al. 2003; Pollak et al. 2004; Hyun et al. 2009; Kim-Eun et al. 2010; Thinh et al. 2013; Han et al. 2015b; Vishchuk et al. 2016). Various researches have examined the impacts of seaweeds on cell death pathways besides apoptosis. The suppression of apoptosis in cancerous cells induces growth and progression of tumor and imparts tolerance to cytotoxic anticancer drugs (Pollak et al. 2004; Hyun et al. 2009; Kim-Eun et al. 2010; Thinh et al. 2013; Moussavou et al. 2014; Han et al. 2015a, 2015b; Somasundaram et al. 2016; Kim et al. 2017).

Various studies of brown algae have revealed that glycoproteins from *Laminaria japonica* (Go et al. 2010) and fucoidans from *Sargassum horneri*, *Ecklonia cava*, and *Costaria costata* (Ermakova et al. 2011) had anticancerous properties on colon cancer cells of human beings. Several studies associated to colon cancer have found that laminarin suppress cancer cells via Fas and IGF-IR signaling through the intrinsic apoptotic and ErbB pathways and promotes Fas-dependent apoptosis by controlling the expression level of Fas and Fas-associated protein with death domain (FADD) (Ji et al. 2012; Park et al. 2013; Ji and Ji 2014).

Fucoidan, an extract from brown algae, is one of the foremost sulfated polysaccharides; and its sulfate group plays a significant role in copious biological activities (Itoh et al. 1993; Chida and Yamamoto 1987; Koyanagi et al. 2003). The structures and compositions of fucoidan differ among varied brown seaweed species, but usually the compound consists mainly of L-fucose and sulfate, together with little amounts of D-galactose, D-mannose, D-xylose, and uronic acid (Bilan et al. 2002, 2006; Kim-Eun et al. 2010). Several earlier reports have revealed that fucoidan exerts antibacterial (Zapopozhets et al. 1995), antiviral (Hayashi et al. 2008), anticoagulant, antioxidant (Wang et al. 2008), anti-inflammatory, and immunomodulatory effects (Zapopozhets et al. 1995; Hayashi et al. 2008). There have also been a range of researches dealing with the anticarcinogenic effects of fucoidan.

Recently, the development of chemoprevention protocols employing natural or synthetic agents for the inhibition of cancer has been recognized (Mann et al. 2005). Consequently, there is a vital requirement for novel chemopreventive agents with least side effects and toxicities. Recently, bioactive compounds derived from natural resources have become the focus of a large amount of notice from researchers seeking to develop chemopreventive agents, due primarily to the potential cancer-preventive and/or therapeutic activities of many of these compounds at nontoxic levels. However, sustained research into the mechanism of action of these compounds will be necessary for realistic assessments of their cancer chemopreventive qualities.

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Chapter 14
Recent Update on Nanomedicine-Based Drug Targeting on Colon Cancer

Vijay Kumar Singh, Veena Singh, and Vishal Tyagi

Abstract Colorectal cancer (CRC) is a major cause of mortality and highly prevalent worldwide. Currently, no effective delivery system is available for clinical use which could specifically target metastatic cancers. Due to the complicated and variable metastasis process, the current treatment methods for colorectal cancer liver metastasis cannot meet the clinical needs. In particular, targeted nano-drug delivery to the colon is advantageous for colon-specific diseases because nanoparticles can accumulate on the surface of tumor cells, improve the efficacies of therapeutics, and enable localized treatments, which reduces systemic toxicity. The nanoparticle-based drug delivery system is one of the most promising strategies for cancer therapy. In this chapter, we summarize the limitations of colon drug delivery, potential targets for colorectal cancer treatment, and challenges faced by colon-targeted delivery systems. Furthermore, this chapter provides an updated summary of recent advances in the development of novel drug delivery system for colon targeting and future advances in this area of research.

Keywords Cancer therapy · Colorectal cancer · Drug delivery · Targeted nanoparticles

Abbreviations

5-ASA	5-Aminosalicylic acid
5-FU	5-Fluorouracil
ABC	ATP-binding cassette
AJCC	American Joint Committee on Cancer
CD	Crohn's disease

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CDDS	Colon drug delivery system
CDs	Cyclodextrins
CRC	Colorectal cancer
FA	Folic acid
GIT	Gastrointestinal tract
HA	Hyaluronic acid
HBsAg	Hepatitis B virus surface antigen
IBD	Inflammatory bowel disease
IL	Interleukin
MDR	Multidrug resistance
MEs	Microemulsions
MRPs	Multidrug resistance proteins
MSNs	Mesoporous silica nanoparticles
NPs	Nanoparticles
PAMAM	PEGylated polyamidoamine
UC	Ulcerative colitis

14.1 Introduction

Colorectal cancer (CRC) is the third leading cause of cancer mortality worldwide, and its incidence is rising gradually in developed countries (Siegel et al. 2014). CRC is usually developed from proliferation of mutated epithelial cells lining the colon, as a result of mutation in Wnt signaling pathway. Mutation of APC gene is the most common genetic defect that leads to high accumulation of β-catenin proteins. The nuclear translocation of these proteins and inappropriate activation of gene transcription cause cancer (Eshghifar et al. 2017). According to GLOBOCAN 2018, in India, the number of new case for CRC was 2.63%, death rate was 2.72%, and 5-year prevalence rate was 53,700 of total cancer incidence. When looking at the USA, the incidence, mortality, and prevalence rates of CRC are consistently high. According to Colorectal Cancer Statistics 2020, it is estimated that there will be 147,950 individuals newly diagnosed with CRC in the USA, including 104,610 cases of colon cancer and 43,340 cases of rectal cancer. However, the majority of cases occur in individuals aged 50 years and older. Approximately, 17,930 new cases will be diagnosed in individuals aged younger than 50 years. Furthermore, it is estimated that there will be 53,200 death cases of CRC in 2020, including 3640 individuals aged younger than 50 years (Siegel et al. 2020). The causes of CRC are not clearly understood; evidence available suggest that the disease develops due to certain risk factors and changes in lifestyle such as physical laziness, bad nutritional habits, obesity, alcohol drinking, and smoking (Islami et al. 2018; Potter 1999). The clinical presentation of CRC patients depends on the size, location, and proximity of metastases. The common clinical symptom includes abdominal swelling and pain, changes in bowel movements, rectal bleeding, and stool mixed with blood

(Del Giudice et al. 2014). Colorectal cancers begin with proliferation of healthy cells in the inner lining of the colon or rectum. These clumps of cells (tumor) are called polyps and it can change into cancerous tumor over time. However, all polyps cannot become cancer; it depends on the type of polyps. There are several types of polyps. Adenomatous polyps may sometimes become cancerous, and it is considered precancerous condition. Hyperplastic polyps are common but not considered as precancerous. Serrated polyps have high risk of colorectal cancer and are often treated like adenomas (Jasperson et al. 2010; Terzić et al. 2010). Most CRC starts in the mucosa and can grow in outward direction through submucosa, muscle layer, or other layers. When cancer cell reached at the outer wall, it can enter into blood vessels. Once they grow into lymph vessels, from there, they can move to the lymph node and other parts of the body. The extent of spread (stage) of CRC depends on the depth of its growth into the wall and outside of the colon. The stage of cancer defines the extent of cancer spread in the body. In addition, it helps in diagnosing the severity of the disease and in deciding the best treatment approach (Lee et al. 2019). According to the American Joint Committee on Cancer (AJCC) guidelines, CRC can be classified into four stages (Lee et al. 2018). The earliest stage of CRC is called stage 0. The rate of survival at early stages (0 to IIC) is high above 50%. Up to this stage (IIC), cancer has grown on the wall of the colon or rectum, but it has not spread to nearby tissues or organs. The later stages (IIIA to IVB) are diagnosed as advanced stages and show very low survival rate below 5%. In these stages, cancer cells spread to more than one distant organ such as the liver or lung (Weiser 2018; Tian et al. 2018). In advanced stages of CRC, effective therapeutic strategy is required to diagnose and improve the life expectation of patients (Bennedsgaard et al. 2020; Lee et al. 2015; Nordlinger et al. 2005).

14.2 Conventional Therapy for Colon Cancer

The currently available treatment modalities for CRC are surgical operation, radiation therapy, and chemotherapy (Tang et al. 2015). Very recently, molecular targeted therapies (immunotherapy) are introduced as treatment option for colon and rectal cancer. The selection of treatment option depends upon the stage of cancer, and sometimes two or more treatment options are applied at the same time (You et al. 2016). Among the available treatment modalities for CRC, the ideal one may be considered on the basis of their ability to prevent the occurrence of cancer. It is assumed that chronic inflammation associated with inflammatory bowel disease (IBD) may increase the risk of CRC. So, the drugs used for the treatment of Crohn's disease and ulcerative colitis (IBD) may be helpful to reduce the possibility to develop CRC (Rubin 2008). For instance, 5-aminosalicylic acid (5-ASA) is an anti-inflammatory drug usually used to treat inflammatory bowel disease. Moreover, it has been investigated for anticancer activity, and it is capable to lower the CRC occurrence (Lyakhovich and Gasche 2010).

Chemotherapy is potential therapeutic approach that utilizes different drugs alone or in combination to decrease the cancerous cell division. The conventional therapies available for treatment of CRC are not site specific and result in unwanted side effects, for instance, gastrointestinal toxicity, hematologic disorders, liver toxicity, anemia, and hand-foot syndrome.

Till date, no satisfactory results have been achieved; however, the associated side effects lead to the development of drug resistance (Ades 2009; Son et al. 2009).

Most anticancer agents do not kill the cancerous cells specifically. They are unable to differentiate normal and cancerous cells and produce severe side effects in other tissues. These systemic toxicity and adverse effects limit the maximum systemic dose and lead to inadequate drug supply at the tumor site. The strategies used for colon-targeted drug delivery include use of pH-sensitive polymer, slow-degrading polymer, prodrugs, and polysaccharides. However, these approaches lack site specificity and uniformity in release profile. Even some of them continuously releasing drug in GIT before reaching the colon, which results in less drug availability at disease site and more side effects (Zhang and Merlin 2018). The reasons for variation in therapeutic efficacy may be the changes in physiology, pH of GIT, and individual colon microbiome (Hebden et al. 2000; Talley et al. 2011). Several investigations have been carried out for direct drug delivery to the colon, but due to the large size of conventional formulations, they were unable to penetrate the mucus layer and disrupt the colitis tissues. Therefore, direct drug delivery approaches were ineffective to deliver drug in the entire area of the colon and are not a preferable strategy for the treatment of CRC (Malayandi et al. 2014). These limitations of conventional therapeutic strategy suggested that there is a need for designing an advance drug delivery system with improved drug targeting efficacy, less side effects, and maximum therapeutic outcomes.

Nanotechnology is an emerging technology and offers several advantages over conventional drug delivery systems. The delivery of anticancer agents by using nanocarriers helps to overcome several side effects. At present, nanocarrier drug delivery systems have gained more attention of researchers due to their physicochemical properties and biocompatibility in biological conditions. Nanoparticles play a vital role in targeted drug delivery by covalent bonding with cancer receptors. It can target the anticancer drugs to tumor sites and enhance the drug efficacy, thereby decreasing the side effects. Nanocarriers are polymer- or lipid-based drug delivery system and globally recommended to identify cancerous cells and treat with less side effects. In particular, targeted nano-drug delivery to the colon is advantageous for colon-specific diseases because nanoparticles can accumulate on the surface of tumor cells, improve the efficacies of therapeutics, and enable localized treatments, which reduces systemic toxicity. The nanoparticle-based drug delivery system is one of the most promising strategies for cancer therapy. In this chapter, we summarize the limitations of colon drug delivery, potential targets for colorectal cancer treatment, and challenges faced by colon-targeted delivery systems. Furthermore, this chapter provides an updated summary of recent advances in the development of novel drug delivery system for colon targeting and future advances in this area of research.

14.3 Limitation for Colon Drug Delivery

The drugs which are supposed to assimilate into a colon drug delivery system (CDDS) should have the following physicochemical properties. These drugs should exhibit absorption in upper GIT and show action in the colon for treatment of disease (Talley et al. 2015; Malayandi et al. 2014; Leuva et al. 2012; Das et al. 2010). Moreover, CDDS is associated with certain limitations and challenges because the colon is located in the distal part of the GIT. After the oral administration of drug, it has to travel through the alimentary canal and reach to the site of action. The physiology of GIT is very complex due to pH variations, fluid volume, GI transit times, metabolic enzymes, and presence of food. These factors can be considered as major obstacles for efficient drug delivery to the colon. The solubility and stability of drug in the colon can also be a matter of major concern in drug delivery. The low luminal fluid volume, high viscosity, and neutral pH can affect the solubilization and alter the colonic absorption of drug. Furthermore, nonspecific interaction of drug with intestinal secretions, fecal matter, and dietary residue can influence the stability of drug (Das et al. 2010). Additionally, the degradation of drug due to colonic bacterial enzymes can also make it ineffective.

There are several factors which may influence the bioavailability of drug through CDDS. Therefore, some of these physiological and pathological factors that play important role in designing of drug delivery system to increase efficacy are discussed below.

14.3.1 Anatomical Factors

The large intestine is divided into three parts, i.e., the colon, rectum, and anal canal, and it is approximately 150 cm long. The right colon consists of the appendix, cecum, ascending colon, hepatic flexure, and half part of the transverse colon. The left colon consists of the sigmoid, descending colon, splenic flexure, and other half part of the transverse colon. The anatomic segment rectum is the last part before the anus. The physiology of the colon is different from other parts of GIT. Furthermore, physical properties and physiology of anatomic parts of the colon are also different (Coupe et al. 1991; Dressman et al. 1990). The main function of the colon is to create appropriate environment for the growth of bacterial enzymes and work as reservoir for colonic contents. The absorption capacity of the colon is also very high, and about 90% of the fluid that enter into the colon are absorbed (Bajpai et al. 2003). The variation in movement of food and drug, across the colon is a major challenge for development of CDDS. Variations in the pH of GIT, fluid volume, viscosity, and microbial enzymes have been observed among various disease states, fed or fast states, ages, and sex in humans (Ibekwe et al. 2008; Rubinstein 1990). These physiological factors influence the bioavailability and drug delivery efficiency of CDDS.

14.3.2 Intestinal Transit Time

The colonic transit time is an important factor that needs to be considered to maximize the efficiency of CDDS and bioavailability of drug. Transit time of drug in the colon is highly variable, and based on different transit time, several CDDS have been developed (Wei et al. 2008; Maroni et al. 2009). The delivery systems are designed in such ways that they release the drug in predetermined time. The colonic disease states significantly influence the transit time. For instance, in case of diarrhea, transit time will increase, while in constipation, it will decrease (Rao et al. 2004). Patients with ulcerative colitis (UC) have faster transit time, but in Crohn's disease (CD), the transit time was reduced compared to the healthy patients (Hebden et al. 2000; Rana et al. 2013). Furthermore, colonic transit time of drug varies according to the dosage form. Generally, it depends on the time of administration, type of dosage form, and presence or absence of food. The transit time for tablet dosage across GIT is 5.8 hours, pallets move faster than tablet and its transit time is 4.6 hours, solution moves in 4.4 hours, and the transit time for capsules is 4.0 hours (Kothawade et al. 2011). Researcher suggested that smaller particles have delayed transit time than larger particles (Stubbs et al. 1991). The above fact suggested that therapeutic efficacy of drug delivery systems can greatly influence the transit time. So it needs to be considered while designing the CDDS.

14.3.3 Colonic Fluid Volume and Viscosity

The major function of luminal fluid is to dissolve the drug and enable its absorption by bringing them into contact with GIT absorptive tissues. Hence, the availability and distribution of this fluid is required for effective drug delivery. However, fluid volume in GIT is highly dynamic and results in the secretion, absorption, and transport of contents. Generally, human GIT handles 8–10 L of fluids daily, among these 2–3 L comes from food and water intake, and the major part is from digestive juice (Jequier and Constant 2010). The colon has high water-absorbing capacity, and every day approximately 1.5 L of fluid enters the colon. About 90% of fluids are absorbed by the colon and 200 ml are excreted through feces. This daily turnover of fluids in GIT definitely influences the drug absorption (Sandle 1998; Schiller et al. 2005). Hence, dissolution of drug from delivery system becomes challenging due to the low level of colon fluid and affects its local bioavailability.

The viscosity of colonic contents is high due to the high water-absorbing capacity. Subsequently, it can affect drug dissolution and contact of drug to mucosal wall for absorption (Nunes et al. 2014). Moreover, an increase in the viscosity of colonic contents increases the transit time. Hence, it is important to consider the dosing time of drug with respect to the bowel movement (Sathyan et al. 2000). The frequency of bowel movement can be varying in diarrhea which decreases the retention of dosage form and drug release. These factors should be considered while designing delivery system to ensure the efficacy of CDDS.

14.3.4 Colonic pH

The pH variations at different regions of GIT are challenging to design an effective drug delivery system. The stomach has highly acidic pH range from 1 to 3, whereas the small intestine has slightly acid to neutral pH range from 6 to 7.5 and the colon has a pH range from 5 to 8 (Collnot et al. 2012; Koziolek et al. 2015). The pH of the stomach, intestine, and colon may be influenced by diet, food intake, and disease state. The pH of the colon may reduce due to carbohydrate-rich diet because polysaccharides are known to be fermented by colonic bacteria and produce lactic acid (Macfarlane et al. 1992; Nugent et al. 2001). The disease states of GIT have also been changing the pH of the colon. For instance, low pH values in the colon have been found in inflammatory bowel diseases. In Crohn's disease patient, the pH of the small intestine has been found unchanged, but the pH of the colon has been significantly reduced compared to healthy person (Fallingborg et al. 1998). The above fact suggested that the efficacy of pH-dependent CDDS is probably affected by low pH of the colon. These changes in colonic pH influenced the solubility of drug and result into changes in pharmacokinetic and pharmacokinetic profile of CDDS. In drug delivery point of view, the development of pH-dependent CDDS is a potential strategy because degradation of drug and carriers in acidic environment of stomach can be avoided by coating with polymers which are stable at acidic pH (Mrsny 2012; Kim et al. 2018).

14.3.5 Colonic Enzymes and Metabolism

The human colon is consisting more than 400 different species of aerobic and anaerobic bacteria of *Clostridium* and *Escherichia coli* species (Gibson et al. 1989). These bacteria contain several metabolic enzymes, which catalyze the metabolism of drugs, biomolecules, inactivation of toxic metabolites, and fermentation of carbohydrates and proteins (Chung et al. 1978). The drug releases at different parts of GIT are depending on the presence of bacterial enzymes and microflora. These enzymes are able to break the bond between carrier and active drug that results in the release of drug from the formulation. Polysaccharides are commonly used as carrier or drug release controlling agents in CDDS and are only metabolized by anaerobic bacteria residing in the colon (Guarner and Malagelada 2003). The drugs are also susceptible to early metabolism by colonic enzymes. However, metabolites produced by the reaction between drug and colonic enzymes are pharmacologically active and sometimes toxic (Guarner and Malagelada 2003; Kang et al. 2012). In addition, these metabolic actions of enzymes are influenced by GIT disease, drug therapy, and dietary residue and lead to inactivation of drug (McConnell et al. 2008). Researcher suggested that load of colonic microflora is affected by CD and UC (Linskens et al. 2001). The above facts suggested that such condition can alter the drug release from enzyme-dependent formulation and should be considered during designing the CDDS. The prodrug approach which is based on formation of active metabolite by metabolism of drug is commonly used for CDDS.

14.3.6 Multidrug Resistance as Barriers

Multidrug resistance (MDR) is causing chemotherapy failure, and it occurs when cancerous cell resists to multiple therapeutic agents which differ structurally and functionally (Lage 2008). The mechanisms of MDR involve decrease in drug uptake, increase in drug efflux, impaired apoptosis, and changed cell cycle. In order to improve the efficacy of treatment modalities for CRC, it is important to understand the biological mechanism of MDR. The hypothesis of MDR is due to the overexpression of ATP-binding cassette (ABC) membrane transporters, i.e., multidrug resistance proteins (MRPs) and P-glycoprotein (P-gp), that efflux out the chemotherapeutic drugs from cancer cells (Kathawala et al. 2015). Several studies suggested the high expression of MRP1 belonging to MRP family has been associated with acquired chemoresistance in CRCs; however, its role in MDR of phenotype of CRC remains unclear (Ji et al. 2014; Xing et al. 2014; Micsik et al. 2015). Therefore, further studies are required to clarify the role of MRP1 in the CRC-associated MDR.

14.4 Targets for CRC Treatment

14.4.1 Receptors and Transporters

Cancer cells replicate more frequently as compared to normal cell and express certain type of receptors and transporters on their surface (Hanahan and Weinberg 2000). The conjugation of ligands such as peptides, vitamins, hormones, and specific antibodies on the surface of drug delivery system makes an efficient drug targeting approach (Moghimipour et al. 2018; Amin et al. 2015; Liu et al. 2019). For instance, folic acid (FA) receptors are overexpressed on CRC. Therefore, FA-conjugated delivery systems are likely to enhance the uptake of drug by cancer cell (Farran et al. 2020; Handali et al. 2019). In Caco-2, CT26, and HT-29 cells, FA-liganded 5-fluorouracil showed increased cellular uptake as compared to free 5-fluorouracil with 3 times less IC50 and 25 times more production of ROS. Furthermore, in vivo studies demonstrated that FA-conjugated liposome formulation inhibited significantly higher tumor growth as compared to the free drug. In addition, biocompatibility and safety margin of FA receptor-targeted drug delivery approach were established by hematological and histological studies (Moghimipour et al. 2018; Handali et al. 2019). The biopsy study on human colon specimen showed presence of amino acid and peptide transporter, for example, PepT1 and CD98 in CRC patients. The above findings suggested that these transporters may be used as potential therapeutic targets for drug delivery. These findings were further confirmed by using CD98-functionalized nanoparticles to deliver camptothecin, an anticancer drug to CRC cells (Yang and Merlin 2019; Xiao et al. 2018). CD98-guided nanoparticles showed excellent anticancer activity as

compared to other nanoparticles. Further studies are required to establish that amino acid transporters are potential targets for drug delivery and treatment of CRC.

14.4.2 Colon Cancer Stem cells (CCSCs) Target

The emerging evidence suggested that colon cancer stem cells are critical to eliminate in CRC (Das et al. 2020). Recently, the concepts of cancer stem cells were found prevalent and having abilities to self repair, differentiate and give rise to all type of cells within tumor of colon cancer. CCSCs were considered a major driving force for the initiation and propagation of metastases, recurrence of tumor, and development of resistance against conventional chemotherapy (Croker and Allan 2008; Botchkina 2013; Gener et al. 2015). At present, researchers are paying more attention to develop suitable techniques for isolation and identification of cancer stem cells. These studies will help to not only understand treatment failure but also provide CCSC-based therapeutic strategy to cure cancer. Some CCSCs can be characterized by biomarkers expressed on cell surface, for example, CD24, CD44, CD133, CD166, Lgr5, and EpCAM (Chaitra et al. 2019; Patel et al. 2009; Morral et al. 2020; Watanabe et al. 2017). The therapeutic approach targeting one of these or more biomarkers can offer effective drug targeting CCSCs. CD44 biomarker is a glycoprotein that binds with hyaluronic acid (HA) in primary tumor cells and metastasis-initiating cells (Debele et al. 2018). HA-conjugated nanoparticles that target CD44 on metastasis cells have been reported for targeted delivery of anticancer drugs. B. Mansoori et al. developed an HA-conjugated liposome that encapsulated 5-FU (5-FU-HA) with an average particle size of 144 ± 77 nm. The developed formulation was applied on a CD44-expressing HT-29 CRC cell line and a non-CD44 expressing hepatoma cell line (Mansoori et al. 2020). The results show that 5-FU-HA demonstrated high cellular uptake and 5-FU release into HT-29 cells, but the uptake and release of 5-FU in hepatoma cells is minimal. These findings suggested that 5-FU-HA have optimal targeted 5-FU delivery into colorectal cancer cells.

14.4.3 Colorectal Cancer Microenvironment

The development of CRCs involved multistep process which starts from normal epithelium cell to polyp and finally to invasive carcinoma supported by tumor microenvironment. The tumor microenvironment includes tumor-infiltrating cells, extracellular matrix, stromal cells, immune cells, secreted factors, signaling molecules, and surrounding blood vessels (Li et al. 2020; Gil et al. 2018; Pascussi et al. 2016). The microenvironment of the cancer appears to be playing a key role in the growth, metastases, and drug resistance of the colorectal cancer. It constitutes tumor-associated fibroblasts, an extracellular matrix capable of promoting

carcinogenesis and decreasing therapeutic efficacy, regulated immune cells, and tumor vasculature. Such components interact with cancer cells via a collection of secreted molecules that support paracrine signaling, as well as cell-to-cell communicating mechanisms, producing a bi-univocal communication array. In general, signals arising from colorectal cancer stromal cells are used to activate different gene expression profiles in cancer parenchymal cells that sequentially induce deformed gene expression profiles in stromal cells (Fuhr et al. 2019). The altered epithelial cells tune the function of stromal cells to favor its own growth, survival, and metastasis. The signaling molecules, for example, ROS, can act as essential microenvironmental indicators and regulators for uncontrolled cell growth and metastasis. In CRC growth, secreted factors support the generation of new blood vessels (angiogenesis). This dependence of cancer cells to the microenvironment components opens new possibilities for the development of potential therapeutic agents for the treatment of CRC (Wu et al. 2019; Fernandes et al. 2018). Recently, X. Duan et al. developed lipid-based nanoparticles for effective delivery of an immunostimulatory chemotherapeutic combination of oxaliplatin and dihydroartemisinin that changed the microenvironment of CRC (Duan et al. 2019). The delivered drugs showed enhanced ROS generation which activates immune responses with anti-PD-L1 antibody to treat CRC. The use of lipid-based nanoparticles considerably improves the biodistribution and drug uptake by tumor. Additionally, the developed nanoparticles reduce the risk of peripheral neuropathy associated with encapsulated drug and activated the natural immune system and therefore bring long-lasting antitumor immunity (Duan et al. 2019; He et al. 2016). After repeated dosing of formulation, the recurrence of tumor was prevented and all tumors were ultimately eliminated.

Increasing evidence provides understanding the vital aspect of microenvironment in regulating abnormal tissue formation, tumor progression, production of localized resistance to chemotherapy, and metastases. Generally, microenvironment plays a vital part in increasing the therapeutic efficiency of chemotherapy in clinical oncology. Furthermore, its play an important role in enhancing the nanosystems to evoke the immunity of the antitumor areas, which may be useful in the treatment of cancer (Fernandes et al. 2018). Shojaee et al. proposed drug-coated magnetic nanoparticle to control the cancer microenvironment. Three factors – size of the magnetic nanoparticle, the magnetic field strength, and the distance between the tumor and the magnet – are recommended for their effects on the delivery of drugs to the tumor tissue. The results revealed moderate effect on penetration of drug to cancer tissues by the magnetic field and size variations. This mild impact is due to the high density of extracellular matrix and the lower level of interstitial fluid pressure resulting in just a 36% increase rate in drug penetration into the tumor tissue with 10 nm MNP and less than 2.5 T magnetic field strength. Also results show this research helps to understand the magnetic impact on the delivery rate as well as resolve the microenvironment of cancer (Shojaee et al. 2020).

14.4.4 Targeting Colorectal Cancer and Metastatic Liver Cancer

Initially, Paget defined metastasis in his hypothesis of "soil and seed." It is a composite multistep procedure involving the spread of cancer cells to distant organs. The liver is the primary site of metastatic cancer, especially colorectal cancer in which it appears to be a significant cause of cancer-related demise. The liver is a key metabolic organ, and therefore, its complex architecture serves features like protein biosynthesis, host protection, and detoxification of endobiotics and xenobiotics. The features of the liver like its anatomical position and histological architecture make it more susceptible to metastasis (Arshad et al. 2020). Nearly 50% of patients appear to have liver metastases during their illness. Mainly, the liver filters the urine and is a main metabolic site. Therefore, most intravascularly distributed lipid nanoparticles gather in the liver tissues, ensuring that they demonstrate sufficient circulation period and a suitable size (Samuelsson et al. 2017).

Nanotechnologies have become extremely important as systematic methods to cancer diagnosis and therapy. Under this context, the use of polymer-based nanoparticles as selective drug delivery systems is considered to be one of the most promising methods for cancer treatment. Nanoplatforms are useful drug delivery mechanisms that are shown to achieve the dual purpose of enhancing diagnostic accuracy and therapeutic efficacy for colorectal cancer and metastatic liver cancer. It is expected that agents designed to selectively enter the liver and remove metastases would provide a greater survival advantage compared to conventional chemotherapy, which is only moderate in efficacy. As a metastatic site, the liver presents major challenges that influence drug delivery and eventual efficacy. An antimetastatic nanotechnology-based approach may therefore provide a promising route to resolve these issues (You et al. 2016; Arshad et al. 2020).

14.4.5 Nanoparticle-Mediated Gene Therapy

Cancer gene therapy involves two approaches: (a) silencing or deleting cancer genes and (b) activating the immune system to recognize and eliminate CRC cells (Garcia et al. 2020; Duzgunes and de Ilarduya 2012). However, gene therapy has great potential for advanced recurred CRC; major difficulties remain as poor selectivity in targeted delivery system and inefficient gene transfer (Belfiore et al. 2018; Hatakeyama 2018). In general, the effectiveness of treatment is based on how precisely the carrier system delivered the nucleic acid at target site without interfering with the healthy cell.

Virus gene therapies are the most popular and occasionally still in use to deliver gene into the human cells. However, it requires precise manufacturing condition because any trace of impurities causes serious adverse reactions. Therefore, nonviral gene delivery approaches have been slowly replaced the viral gene therapy

(Caracciolo and Amenitsch 2012). Presently, lipid- and polymer-based nanocarrier systems are commonly used for gene delivery because they offer several advantages such as ease in manufacturing and comparatively are safe and flexible in designing (Belfiore et al. 2018). The liposomes were first carrier system used for delivery of nucleic acid. The positively charged lipids were used to encapsulate the negatively charged nucleic acid in liposomes, and by electrostatic attraction, a stable complex has been formed. These stable complexes enter the cell by endocytosis, and when the lipids fuse with cell membrane, nucleic acid is released (Kaneda 1996; Zhang et al. 2019). The stability of these complexes in biological system is a major concern limiting the clinical application of liposomes. Various methods have been developed to increase the stability of liposomes by altering the surface charge. For example, T. Wang et al. developed cationic liposomes by using dioctadecyl-dimethylammonium chloride (DODAC), distearoyl-phosphatidylcholine (DSPC), N-palmitoyl-sphingosine-1-succinyl (PEG-CerC16), and cholesterol. Survivin siRNA was effectively encapsulated in constructed nanoliposomes, and PEG was used to modify the outer layer and increase the stability in circulation. The siRNA/cationic liposomes considerably inhibited the CRC cell proliferation (Wang et al. 2017). The above study suggested that lipid nanoparticles can be used for delivering nuclear acids in colon cancer treatment.

Several studies suggested that surface-modified nanocarriers with antigens and encapsulated antigens can be used to activate the desired immunologic response and showed an excellent therapeutic effect (Kwong et al. 2013). For example, H. Guan et al. reported that the human mucin-1 peptide (BP-25) encapsulated liposomes to activate T-cell-specific response after subcutaneous delivery (Guan et al. 1998). Furthermore, modification on surface charge and constituents of the lipid bilayer of liposomes can also induce specific immune responses. K.S. Kim et al. constructed solid lipid nanoparticles of docetaxel by coating with an anionic polymer conjugated with glycocholic acid. The developed DSLN-CSG inhibited the growth of colorectal adenocarcinoma in animal model. The population of cytotoxic T cells has been increased after the treatment, but the tumor-associated macrophages and regulatory T-cell population have been decreased (Lim and Kim 2002). These findings were suggested that antigen-conjugated lipid-based nanomedicines can be used as a potential approach to regulate the specific immune response and prevent the recurrence of tumor in CRC.

14.5 Colon-Targeted Novel Drug Delivery

14.5.1 Polymer-Based Nanoparticles

Several studies have been suggested that polymeric nanoparticles are effective for the treatment of cancers. The nanoparticles (NPs) developed by using natural polymers and proteins have been found to be effective in CDDS (Ma and Williams 2018;

Zeeshan et al. 2019). Mutalik and coworkers have developed curcumin nanoparticles by using pH-sensitive polyacrylamide-grafted xanthan gum for colon-targeted drug delivery. The drug release from nanoparticles in acidic pH 1.2 and 4.5 was minimal, whereas at neutral pH 7.2, the drug release was rapid and higher (Mutalik et al. 2016). In another work, Sahu and Pandey developed hepatitis B virus surface antigen (HBsAg) for colonic immunization. The HBsAg-loaded NPs were prepared by using the combination of Eudragit L100 and S100, which confirmed the effective distribution of NPs in the colon with improved immune response (Sahu and Pandey 2019). Furthermore, budesonide-loaded nanoparticles have been prepared by using Eudragit FS30D and RS100 with solvent evaporation method. Eudragit FS30D and Eudragit RS100 are pH-dependent and time-dependent polymers, respectively. These pH-/time-dependent nanoparticles reduce the premature drug release and improve the site specificity into the colon (Naeem et al. 2015). In another work, hyaluronic acid (HA)-functionalized polymeric nanoparticles of curcumin and camptothecin (CPT) have been prepared for targeted drug delivery against colon cancer. The NPs showed simultaneous sustained release of both drugs. The cellular uptake study suggested that fictionalization of NPs with HA improves the colon cancer-targeting capability and cellular uptake efficiency compared to the other polymeric coated NPs (Xiao et al. 2015). Malhotra et al. developed polymer-coated chitosan NPs which are functionalized with cell-targeting peptide (CP15). The prepared NPs were delivered intraperitoneally to examine the drug delivery efficacy, cytotoxicity, and biodistribution in colorectal cancer. The results suggested that peptide-functionalized NPs were suppressing the expression of proto-oncogenes (Malhotra et al. 2013). Furthermore, Li et al. prepared RNA aptamer-functionalized curcumin-loaded lipid polymer NPs for targeted drug delivery to colon cancer cells. The results revealed that aptamer-functionalized curcumin NPs have improved drug delivery to colon cancer cells (Lei et al. 2014). Hence, polymer-coated NP drug delivery approach is having potential to increase the efficacy of drug at tumor site and reduce the side effects.

14.5.2 Lipid-Based Formulations

Liposomes were the first nanocarrier system for efficient drug delivery and approved by FDA for application in human (Pattni et al. 2015). Liposome nanoparticles can be prepared by using different phospholipids and cholesterol. Liposomes are biocompatible, biodegradable, and capable to incorporate both lipophilic and hydrophilic drugs (Yoon et al. 2019; Lee 2019). Liposomes contain lipid bilayer in which cancerous cell-specific ligands can be loaded easily and enhance the site-specific drug delivery in cancer treatment (Loira et al. 2014). The liposomes can be coated with pH-dependent polymer to improve the stability in acidic conditions. At present, several liposome-based drug delivery systems have been developed for the treatment of colon cancer. Zhao et al. developed glycol chitosan and Eudragit S100-coated liposomal formulation for colon drug delivery. The developed liposomes were stable in acidic and neutral pH and enhanced the delivery of sorafenib in rat

(Zhao et al. 2018). Moreover, oxaliplatin-encapsulated liposomes (Yang et al. 2011), curcumin, and doxorubicin-loaded liposome (Cay et al. 1997) for colon drug delivery are under preclinical development. Solid lipid NPs were also developed and found superior in terms of entrapment efficiency and extended drug release at target site (Gupta et al. 2017; Pokharkar et al. 2018). Zhang et al. developed folate-modified self-microemulsifying drug delivery system containing curcumin, coated with Eudragit S100. The curcumin-loaded formulation was selectively bound with folate receptors and efficiently delivered curcumin on colon cancer cells (Zhang et al. 2012). In addition, magnetic liposomes exhibit promising properties of targeted drug delivery for colon cancer treatment. 5-FU is an ideal candidate for this system, and to improve the blood circulation time, liposomes were coated with PEG (Ashish et al. 2010). Koning et al. prepared liposomes of 5-fluoro-2′-deoxyuridine that are functionalized with monoclonal antibody. The results suggested that functionalized liposomes improve target efficiency and increase anticancer activity against CRC in animal model (Koning et al. 2002).

14.5.3 Ligand-/Receptor-Mediated Drug Delivery System

Ligand-/receptor-mediated drug delivery systems are more effective for colon cancer with low side effects. These systems are explored to improve the target selectivity by specific interaction between ligands on surface of carrier system and receptors located at cancer cells (Si et al. 2016). Folic acid, hyaluronic acid peptides, and antibodies are used as ligands to design the drug delivery systems, and selection of ligand is based on specific receptor expressed at targeted organ. Several ligand-based drug delivery systems for colon targeting have been developed; some of them are described here. Harel et al. developed anti-transferrin receptor antibody-conjugated liposomes. These antibody-conjugated liposomes demonstrate improved cellular internalization compared to unconjugated liposomes. Moreover, antibody-conjugated liposomes showed selectively more accumulation at inflammation site (Harel et al. 2011). Xiao et al. also prepared NPs conjugated with CD98 antibodies for colon delivery. CD98 is a neutral amino acid transporter expressed in colonic epithelial cell. The surface-modified NPs showed higher affinity with CD98 expressed cells (Xiao et al. 2014).

Folic acid is a tumor selective targeting ligand because folate receptors are highly expressed on cancer cell surface (Shia et al. 2008). Several studies have revealed that NPs functionalized with folic acid can improve the drug uptake selectively on tumor. For instance, folic acid-conjugated liposomes enhanced the anticancer activity of daunorubicin by receptor-mediated drug uptake (Xiong et al. 2011). Handali et al. also prepared folic acid-liganded liposomes of 5-fluorouracil. 5-FU-encapsulated liposomes showed high cytotoxicity and reduce the tumor growth as compared to the plain drug (Handali et al. 2018). The above findings suggested that folic acid-conjugated liposomes are efficient drug carrier and can improve the drug delivery in cancer treatment.

Hyaluronic acid is a disaccharide and has affinity with CD44 receptor expressed in cancer cells. HA-conjugated drug carrier systems are also assessed for selective target drug delivery (Yu et al. 2013; Liu et al. 2015). For instance, HA-conjugated NPs of budesonide were developed for colon-targeted drug delivery. The prepared NPs showed high uptake on inflamed cell containing CD44 receptors (Vafaei et al. 2016). In another work, HA-conjugated PEGylated carbon nanotube containing gemcitabine has been prepared for colon cancer targeting (Prajapati et al. 2019). The result suggested that HA-conjugated drug delivery systems are efficient for cancer targeting. The above finding suggested that HA-conjugated NPs are potential targeted drug delivery for colon cancer treatment. Peptide ligands also showed specificity and high binding affinity with receptors (Al-azzawi and Masheta 2019). Ren et al. examined the applicability of synthesized peptide for colon delivery of anticancer drugs. These peptides were conjugated with doxorubicin-loaded micelles as a ligand. The conjugates showed strong cytotoxicity and effectively enter into tumor cells (Ren et al. 2016). The above finding suggested that peptides are potential targeting ligand for colon drug delivery.

14.5.4 Magnetic Microcarrier for Drug Delivery

Magnetic microcarrier is a promising novel drug delivery system for controlled and target-specific drug delivery. Grifantini et al. developed an NDDS having magnetic properties for efficient drug delivery to colon cancer cell by specific monoclonal antibody. It was observed that this delivery system was efficient for targeting colon cancer cell and reduces cancer cell growth at low dose (Grifantini et al. 2018). The finding of the above study suggested that magnetically driven delivery system can improve drug bioavailability, therebyopening a new way for colon-targeted drug delivery. In another study, the efficacy of hydrocortisone was improved by using magnetic belt. This device contains magnetic porous hydrocortisone-loaded silica microparticles, and the surface of nanodevice was functionalized with urea derivatives. This nanodevice remains closed at neutral pH but opens and releases the payload in the presence of sodium dithionite, a reducing agent for azo moiety (Teruel et al. 2018). Furthermore, Kono et al. also developed magnetically driven cell delivery system of murine macrophage cells. They further confirmed that delivery system can improve colon delivery (Kono et al. 2019).

14.5.5 Micelles

Micelles are spherical-shaped amphiphilic colloidal particles having hydrophilic shell (water soluble) and hydrophobic core (drug payload) (Jasperson et al. 2010; Terzić et al. 2010). They are used as colloidal suspension and considered great interest due to their possible use in both cancer therapy and diagnosis. Such particles

already proved their potential to convey poorly water-soluble chemotherapeutic drugs for improving stability of drug and excellent penetration and also for improving therapeutic efficacy (Lee et al. 2019). Cancer treatment could be failed due to the accumulation of stemlike cancer cells which are resistant to traditional chemotherapy, and micelles provide the most revolutionary strategy to manage these issues. Micelles have major advantages like enhanced retention and permeability and are biodegradable as well as biocompatible for administering chemotherapeutic drugs to colon cancer. Several studies have been conducted regarding the use of micelles against colon cancer (Lee et al. 2018). Weiser used quercetin as a drug against colon cancer, but quercetin is hydrophobic in nature and has low hydrophilicity that obstructs its clinical use in cancer treatment. Therefore, polymer micelles were used for encapsulating the quercetin drug. Polymer micelles may accelerate the dissolution of quercetin in water. In result, quercetin-loaded nanomicelles were developed with an increased drug load of 6.85% and a small particle size of 34.8 nm, fully dispersed in water and delivered in vivo and in vitro for a prolonged period of time. However, compared with free quercetin, quercetin-loaded micelles showed better induction of apoptosis and inhibition of cell growth in CT26 cells in vitro. Additionally, better results of quercetin-loaded micelles on initiating cell apoptosis, inhibiting tumor angiogenesis, and regulating cell proliferation have been observed through immunofluorescence analysis. It showed that quercetin-loaded micelles work as a possible candidate against colon cancer (Weiser 2018).

14.5.6 Liposomes

These are small in size and spherical-shaped artificial vesicles that could be manufactured from cholesterol and nontoxic phospholipids. Based on their size and hydrophobic and hydrophilic properties, they can be used as drug delivery systems (Tian et al. 2018). In various reports, they are used to enhance the therapeutic index of current or future drugs by altering the drugs' structure, extending their biological half-life, and minimizing their metabolism or toxicity (Bennedsgaard et al. 2020). It has been found that liposomes were used to enhance existing chemotherapy regimens due to its capacity to increase the solubility of poorly water-soluble chemotherapeutic drugs and can be used for the treatment of breast cancer, lung cancer, colon cancer, etc. (Lee et al. 2015). Liposomes are good candidate to target colon cancer cells and deliver a therapeutic load directly to a colon cancer cell. Many researches stated the use of liposomes against colon cancer (Nordlinger et al. 2005). Handali et al. developed targeted liposomes loaded with 5-fluorouracil in an attempt to optimize the safety and efficacy of drug. The cell lines used MCF-7, HeLa, HT-29, CT26, and Caco-2 for the evaluation of in vivo cytotoxicity. In result, it was found that entrapment efficiency of the liposomal formulation was 39.71%, and the particle size was found to be about 174 nm. It was also demonstrated that targeted liposomes minimize tumor volume compared with free 5-fluorouracil, and it can minimize the effect of cancer in the colon (Tang et al. 2015).

14.5.7 Dendrimers

The burgeoning role of dendrimers for anticancer therapies had already highlighted the significance of certain excellently defined materials as the latest category of macromolecular nanoscale targeted delivery systems (You et al. 2016). Its biocompatibility, retention, as well as delivery have become much more explicated; distinctive derivatives of dendrimer have also been fabricated for enhanced specificity or even functionality, especially with respect to pharmacokinetics as well as targeted drug delivery. Significant advances have been made especially over the past several years in the utilization of dendrimers for diagnostic and therapeutic applications in colon cancer treatment, particularly significant improvement in delivering antineoplastic and contrast agents (Rubin 2008). Numerous studies have been undergoing in context of dendrimers being utilized as nanocarriers for colon cancer therapy. In a recent study by Pishavar et al., PEGylated polyamidoamine (PAMAM) dendrimers have been designed and developed which are conjugated with cholesterol to deliver the immunogene interleukin-12 (IL-12). IL-12 has been considered an ideal tumor immunotherapy agent. In this, the 3 and 5% primary amines of PAMAM had been replaced by cholesteryl chloroformate along with alkyl PEG to transmit plasmid-encoding gene. The characteristics of altered PAMAMs including size, cytotoxicity, as well as efficiency of transfection had been evaluated in cell line colon cancer. The alkyl PEG- and cholesterol-modified PAMAM could have been a promising approach to enhance the expression of IL-12 in cells of colon cancer. Although IL-12's mechanism of action in cancer is indeed regulating both inherent (natural killer cells) and adaptive (cytotoxic T lymphocytes) type immunity, the efficacy of conjugated carrier in delivering IL-12 plasmid seems to need in vivo cancer therapy studies (Lyakhovich and Gasche 2010).

14.5.8 Cyclodextrins

Numerous studies are going on overenhancing the efficiency of anticancer drugs for cancer therapies, and it has been proposed that cyclodextrins (CDs) can indeed be utilized as a significant carrier in improving cancer therapies (Ades 2009). CDs are indeed a class of cyclic oligosaccharides constituted by linkage of six or even more glucose subunits through α-1,4-glycosidic bonds. CDs can indeed be divided into three types based upon their number of subunits of glucose , viz., α-cyclodextrin (α-CD), β-cyclodextrin (β-CD), and γ-cyclodextrin (γ-CD) (Son et al. 2009). Natural α-CD, β-CD, and γ-CD seem to be more resistant than linear oligosaccharides to nonenzymatic hydrolysis. Moreover, human salivary as well as pancreatic amylases do not hydrolyze the α-CD and β-CD. Because of this feature, cyclodextrin-drug conjugates in upper GI tract remain unchanged until they approach the colon. The large microbiome in the colon, particularly *Bacteroides*, splits the CDs by fermentation into small saccharides, thereby providing a quick release of drug (Zhang and

Merlin 2018). Abundant studies have been undergoing in context of CDs being utilized as nanocarriers for colon cancer therapy. In a study, Hosseinifar et al. designed and developed nanogel based on alginate-CD which is pressure responsive to enhance the apoptosis mechanism of 5-fluorouracil (5-FU) in colon cancer cells. The nanogel has been developed by cross-linking the alginate (Al) with β-CDs, and 5-FU has been loaded into the hydrogel as a model drug. The results demonstrated that the nanogels developed are cytocompatible. The encapsulation of drug has also been enhanced, and the release of drug occurs due to intravascular pressure conditions and controlled release is achieved. The apoptotic mechanism has been enhanced when 5-FU has been incorporated and delivered using nanogel (Hebden et al. 2000).

14.5.9 Microemulsions

Microemulsions (MEs) seem to be colloidal carriers which have fascinated attention because of its potential to overcome some of the other limitations of nanocarriers such as drug instability and leakage (Talley et al. 2015). ME is thermodynamically stable and clear dispersion of two immiscible liquids stabilized by the surfactant molecular interfacial film. Because of their contents of surfactants as well as cosurfactants, these give a great penetration enhancement effect with such a small droplet size of 10–100 nm. The emergence of microdomains of varying concentrations in the same single-phase solution allows for the solubilization of both lipophilic and hydrophilic materials (Malayandi et al. 2014). Abundant studies have been undergoing in context of ME being utilized as nanocarriers for colon cancer therapy. In a study, Abdel-Bar et al. designed and developed an oxaliplatin ME to improve the efficacy of drug toward colon cancer cells. The Miglyol 812 has been used as oil phase and Tween 80 with Labrasol as surfactant and cosurfactant. The ME developed is having a particle size of less than 100 nm. The conductivity studies have been performed which showed that the ME could be either oil/water or water/oil emulsion. The in vitro study results demonstrated that release of drug from water/oil ME is controlled over 24 h, whereas for oil/water, it is only 2 h. The results showed that oxaliplatin ME has been successfully developed and water/oil ME showed very good cytotoxicity (Leuva et al. 2012).

14.5.10 Nanoparticles

Combination chemotherapy based on nanoparticles (NPs) has indeed been perceived as an important approach for achieving synergic effect as well as targeted delivery of drugs for colon cancer treatment. A number of chemotherapeutics have been used for cancer treatment. The proportion of administered drugs that further represents the degree of synergism or antagonism has indeed been considered to be

extremely relevant for the treatment outcomes of combination chemotherapy (Das et al. 2010). In addition, chemotherapeutics has specific cellular uptake profiles, pharmacokinetics, as well as systemic distributions that would make dosing or otherwise administration more challenging. Preferably, such challenges can be addressed by delivering various therapeutic agents concurrently utilizing a nanocarrier system. Various nanocarriers have been used for this purpose, like liposomes, dendrimers, and nanoparticles. Of all these nanocarriers, nanoparticles are considered to have better potential (Coupe et al. 1991). Numerous studies have been undergoing in context of dendrimers being utilized as nanocarriers for colon cancer therapy. In a recent study, Dressman et al. designed and developed mesoporous silica nanoparticles (MSNs) for delivery of colchicine (COL) to colon cancer cells. These MSNs have been functionalized using phosphate groups and then loaded with COL, further coating the MSNs with folic acid-chitosan complex for targeting. The nanoformulation developed is MSNsPCOL/CG-FA. The results showed that the antitumor activity of developed NPs has been enhanced as compared to COL alone. A 100% inhibition of cancer cells has been attained. The reduced cytotoxicity of COL has been enhanced using this developed nanocarrier (Dressman et al. 1990). Some of the selected examples of recently studied nanocarrier-based drug delivery systems for the treatment of colon cancer are summarized in Table 14.1.

14.6 Summary and Future Directions

Colon drug delivery system must be capable of overcoming the barriers in GIT, should be target-specific, and should release specific dose of drug at the disease site. In this context, novel drug delivery system offers a great potential to overcome challenges and is efficient for colon-specific disease. Therefore, it is gaining more attention as an efficient strategy of drug delivery over conventional methods of drug delivery. In this chapter, various approaches have been discussed to develop efficient colon-targeted drug delivery system. To develop an efficient colon-targeted delivery system, it is very prerequisite to consider the physiology and disease state of targeted organ. However, the dynamic changes in disease condition make designing of formulation more complicated and finally lead to failure due to lack of specificity. For instance, changes in pH of GIT disable the pH-dependent drug release that results in premature drug release in the organ and causes toxicity. Nanoparticles are having potential to enhance the specific drug targeting and reduce side effects. The development of nanocarrier-based drug delivery for cancer treatment showed considerable advances in preclinical and clinical studies. Surface modification of nanoparticles on the basis of various targeting mechanisms extends the targeting functions. The goal of this modification is to give precise cancer targeting functions to nanocarriers and optimize its biodistribution and pharmacokinetic patterns of encapsulated drugs with minimum side effects. Several nanoparticle-based medicines have their success story in the medical field, for example, nanoparticles of paclitaxel and vincristine for the clinical treatment of GIT cancer. However, there is

Table 14.1 Summary of nanocarrier-based drug delivery systems for the treatment of colon cancer

Drugs	Carriers	Cells	Method	Advantages	References
Dihydroarte misinin and doxorubicin	Liposomes	HCT8/ ADR	Thin-film hydration method	Inhibit growth of cancer cell, increase synergistic effect, targeted drug delivery, accumulation of therapeutic agents, and enhancement of apoptosis	Ibekwe et al. (2008)
Curcumin	Polymeric micelles	CT26	Solid dispersion method	Improve stability, increase cellular uptake, and increase apoptosis induction	Rubinstein (1990)
5-Fluorouracil	Chitosan microsphere	HT-29	Solvent evaporation method	Improved safety profile, therapeutic efficacy, and increase bioavailability	Amidon et al. (2015)
Imatinib mesylate	Liposomes	Colo 320 and HT-29	Simple mixing method	High drug loading, nontoxic, hemocompatible, and low opsonization	Christl and Scheppach (1997)
Doxorubicin and α-TOS	Polymeric micelles	HCT-116	Solvent exchange method	Rapid release of drug, enhancement of therapeutic effect, biocompatible, increase drug loading and entrapment efficiency	Sandle (1998)
Curcumin and doxorubicin	Liposomes	C26	Lipid film hydration method	Evade reticuloendothelial system uptake, accumulation into tumors, and increase the concentration of drug delivery	Schiller et al. (2005)
Doxorubicin	Silica nanoparticles	SW260	Simple mixing method	Increase therapeutic effect, reduce toxicity, enhanced cellular uptake, and improve therapeutic index	Shameem et al. (1995)
Camptothecin and curcumin	Cationic polymeric nanoparticle	Colon-26	Emulsion solvent evaporation method	Enhance intracellular drug concentration, achieve synergistic effect, increase cellular uptake, and improve therapeutic efficacy	Pijper and Discombe (1946)

(continued)

Table 14.1 (continued)

Drugs	Carriers	Cells	Method	Advantages	References
Camptothecin	PLGA nanoparticles	Colon-26	Emulsion solvent evaporation method	Enhance cellular uptake, high efficacy against tumor, and improved therapeutic efficacy	Evans et al. (1988)
5-Fluorouracil	Silica nanoparticles	Colo-205	Mixing method	Improve therapeutic efficacy of drug, sustain release, reduction of side effects, and enhanced uptake	Fallingborg et al. (1989)
5-Fluorouracil	Beta-cyclodextrin nanogels	HT-29	Emulsification method	Cytocompatible, improve biocompatibility, increased drug loading, and controlled release	Macfarlane et al. (1992)
Imatinib mesylate	Niosomes	HCT-116, MCF-7, and HepG-2	Thin-film hydration method	Improve drug efficacy, improve stability, maximum entrapment efficiency, and sustained release profile	Bown et al. (1974)
5-Fluorouracil	Methyl beta-cyclodextrin	HCT-116	N/A	Suppress tumor growth, enhance dissolution rate, improve solubility and drug bioavailability	Nugent et al. (2001)
Metformin	Cubosomes	HCT-116 and Caco-2	Disrupting a cubic gel phase	Improve absorption, biocompatible, and bioadhesive in nature	Gibson et al. (1989)
5-Fluorouracil	Cubosomal nanosponges	N/A	Emulsion solvent diffusion method	Improve patient compliance, penetrability, stability, and enhance formulation flexibility	Rowland (1988)
Docetaxel and curcumin	Nanofibrous microspheres	CT26	Self-assembling method	Self-assembled, biodegradable, and decrease toxicity	Chung et al. (1978)

still shortage of efficient nanomedicine for the treatment of CRC. Diverse shape, structure, and chemical properties of nanoparticles make them one of the safest platforms and capable of encapsulating various types of anticancer drugs. Furthermore, lipid-based nanoparticles are the first nanocarriers that are used with success in clinical trials for CRC treatments. CRC can be considered as a combination of different diseases because different genetic and biological alterations were seen in different patients. Considering these facts, biomarkers identified in CRC and in metastatic cancer cells can be considered as potential targets for treatment strategy of CRC. Moreover, it is revealed that fluctuations in drug plasma concentrations during treatment cycles of chemotherapy can develop drug resistance and result in treatment failure and death of CRC patients. In such complicated situations, selection of appropriate treatment strategy and designing of relevant drug delivery system are quite difficult.

Scientifically, nanomedicines are suitable for treating cancer; however, nanocarriers have flexible drug loading capacity and target selectivity. Lipid-based nanoparticles offer several advantages in terms of particle sizes, adjustable dose, versatile targeting functions, and controlled drug release. In conclusion, the advance understanding of genetics, immunology, and pathophysiology of colon cancer suggested that lipid-based nanoparticle therapy can be used to eliminate CRC. At the same time, further study is required to identify the new targets for anticancer drug in CRC and effectiveness of targeted drug delivery systems.

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Chapter 15
Therapeutic Targeting of Glutamine Metabolism in Colorectal Cancer

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Abstract Colorectal cancer is one of the most commonly diagnosed incurable multifactorial malignancies in the world. To date, there are no promising noninvasive therapeutic tools that have achieved CRC prognosis, survival, and recurrence in clinical settings. We are now very familiar with the most famed term "metabolic reprogramming" that cancer cells preferably employ to meet their rapid bioenergetic and ATP synthesis requirements. Glutamine is the most abundant amino acid in human blood plasma and is known for its significant pleotropic role in the metabolic network.

Here, we exposed the metabolic distortion associated with the metabolism of glutamine in the CRC. Classically, findings have shown that dysregulated glutamine metabolism is significantly associated with CRC growth, survival, metastases, and recurrence. As a result, blocking signaling pathways, enzymes, and transporters associated with glutamine metabolism could be a gold standard strategy to hijack the development of CRC. We hope that this strategy will help to systematically target, manage, and cure CRC.

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Abbreviations

968	5-[3-Bromo-4-(dimethylamino)phenyl]-2,3,5,6-tetrahydro-2,2-dimethyl-benzo[a]phenanthridin-4(1H)-one
ABCA1	ATP-binding cassette transporter A1
ACSL1	Adipose acyl-CoA synthetase-1
AGPAT1	1-Acylglycerol-3-phosphate o-acyltransferase 1
ALL	Acute lymphoblastic leukemia
ALT	Alanine aminotransferase
AOA	Aminooxyacetate
AST	Aspartate aminotransferase
ATP	Adenosine triphosphate
BCH	2-Aminobicyclo-(2,2,1)-heptane-2-carboxylic acid
BPTES	Bis-2-(5-phenylacetamido-1,3,4-thiadiazol2-yl)ethyl sulfide
CAD	Carbamoyl-phosphate synthetase 2, aspartate transcarbamylase, dihydroorotase
CB-839	Telaglenastat
COX2	Cyclooxygenase-2
CRC	Colorectal cancer
DNA	Deoxyribonucleic acid
E2F	E2 promoter binding factor
EAA	Essential amino acid
EAAT	Excitatory amino acid transporter
EGCG	Epigallocatechin gallate
FADH2	Flavin adenine dinucleotide
FASN	Fatty acid synthase
FBP1	Fructose-1,6-bisphosphatase
FDA	US Food and Drug Administration
GABA	Gamma aminobutyric acid
GFAT	Glutamine fructose-6-phosphate amidotransferase
GLN	Glutamine
GLS	Glutaminase
GLU	Glutamate
GLUD	Glutamate dehydrogenase
GLUT1	Glucose transporter 1
GOT2	Glutamate-oxalate transaminase
GPNA	L-γ-Glutamyl-p-nitroanilide
GPT2	Glutamate-pyruvate transaminase
GSH	Glutathione
HIF-2α	Hypoxia-inducible factor-2
HK	Hexokinase

HND	Hartnup disorder
HSF1	Heat shock factor 1
JPH203	(S)-2-amino-3-(4-((5-amino-2-phenylbenzo[d]oxazol-7-yl) methoxy)-3,5-dichlorophenyl
KRAS	Kirsten rat sarcoma viral oncogene
LDH	Lactate dehydrogenase
L-DON	6-Diazo-5-oxo-L-norleucine
MCT4	Monocarboxylate transporter 4
miRNA	MicroRNA
MPC1	Mitochondrial pyruvate carrier 1
mTOR	Mammalian target of rapamycin
NAD	Nicotinamide adenine dinucleotide
NADPH	Nicotinamide adenine dinucleotide phosphate oxidase
NEAA	Non-essential amino acid
NF-kB	*Nuclear factor* kappa-light-chain-enhancer of activated B cells
NH_3	Ammonia
OXPHOS	Oxidative phosphorylation
p53	Tumor suppressor 53
PET	Positron emission tomography
PHGDH	Phosphoglycerate dehydrogenase
PKCζ	Protein kinase C zeta
PKM2	Pyruvate kinase muscle isozyme M2
PPARG	Peroxisome proliferator-activated receptor gamma
PSAT1	Phosphoserine aminotransferase 1
R162	2-Allyl-1-hydroxy-9,10-anthraquinone
Rb	Retinoblastoma
ROS	Reactive oxygen species
SAM	S-Adenosylmethionine
SCD1	Stearoyl-coenzyme A desaturase 1
SIRT4	Sirtuin-4
SLC1	Solute carrier 1
SLC1A5	Solute carrier family 1 member 5
SLC38	Solute carrier 38
SLC38A1	Solute carrier family 38 member 1
SLC38A2	Solute carrier family 38 member 2
SLC38A3	Solute carrier family 38 member 3
SLC38A5	Solute carrier family 38 member 5
SLC38A7	Solute carrier family 38 member 7
SLC6	Solute carrier 6
SLC6A14	Solute carrier family 6 member 14
SLC6A19	Solute carrier family 6 member 19
SLC7	Solute carrier 7
SLC7A5	Solute carrier family 7 member 5
SLC7A6	Solute carrier family 7 member 6
SLC7A7	Solute carrier family 7 member 7

SLC7A8	Solute carrier family 7 member 8
SLC7A9	Solute carrier family 7 member 9
SSZ	Sulfasalazine
TCA	Tricarboxylic acid
TNM	Tumor node metastasis
V-9302	2-Amino-4-bis(aryloxybenzyl)aminobutanoic acids
α-KG	Alpha-ketoglutarate

15.1 Introduction

Colorectal cancer (CRC) is ranked as the third most common form of malignancy and the fourth leading cause of cancer-related death with approximately 1.8 million new cases and 881,000 deaths in 2018 (Bray et al. 2018). Drug resistance and recurrence have a significant challenge in the treatment of cancer, including CRC (Russi et al. 2019; Zare-Bandamiri et al. 2017; Verma et al. 2020a). However, it was expected that the global burden of CRC will increase by almost 60% by 2030. Colorectal malignancy has a significant effect on the quality of life and is a major economic burden on the society and CRC patients (Sun and Zhu 2018; Ferlay et al. 2018).

It is widely accepted that CRC is a multifactorial disease developed by the accumulation of multiple abnormal events, including lifestyle, environmental factors, mutations in anti-proliferative genes (tumor suppressor), and expression of oncogenes (Nguyen and Duong 2018). Studies have shown that CRC is also associated with metabolic changes resulting in a significant number of metabolites (Kong et al. 2018). Recently, few prognostic factors are considered to be a promising survival resource for CRC patients, namely, TNM staging, histological parameter, tumor location, and carcinoembryonic antigen (CEA) rates (Arnold et al. 2017; Zhang et al. 2015).

At present, due to limited treatment choices and inadequate diagnosis, the median overall survival of CRC metastases decreased by 60% with a 5-year survival rate, and this may be more important in the advance stage of the disease due to the late detection and resistance of patients to certain combination therapy (Park et al. 2014). Consequently, due to lack of therapeutic choices and inadequate management, there is an immediate need to create a gold standard compound and therapeutic to cope with colorectal cancer.

Metabolic pathways are fundamental sources of ATP and serve as a recycling plant for the generation/regeneration of macromolecules required for the life of tumor cells. Most cancer biologists have shown that cancer cells have a license to interrupt normal metabolic pathways by metabolic rewiring to replenish their nutritional requirements that are essential to their rapid growth and spread. Metabolic rewiring is caused by the alteration of the expression of various metabolic enzymes, tumor suppressor genes, and oncogenes that redefine the transport and flow of metabolites through the metabolic network to help cancer cells. Warburg effect is the most desirable and recognized hallmark of cancer accompanied by glutamine (GLU) addiction (Yang et al. 2017; Pavlova and Thompson 2016).

Glucose is the primary metabolic food for cancer cells compared to amino acids, usually glutamine, which also acts as a fuel source and contributes to tumor development and proliferation (Scopelliti et al. 2018). Over the last few decades, studies have shown that the reported requirement of metabolic precursors, carbon skeleton, and nitrogen makes cancer cells more dependent on glutamine. Avid intake of glutamine and cancer cell metabolism have also become areas of focus in the exploration of new therapeutic targets in cancer patients in recent years (Luengo et al. 2017). Studies have mainly focused on the diagnostic value of glutamine in the CRC, and minimal studies have examined the prognosis and predictive effects of glutamine (Sirniö et al. 2019; Choi and Park 2018). In the last few years, the glutamine metabolism has gained much attention due to its more consumption by cancer cells in an unusual way, distinct from normal cells. The goal of this chapter is to highlight the role of glutamine metabolism as a potential therapeutic tool for targeting CRC to reduce future burdens in the global context.

15.2 Metabolism of Cancer and Normal Cells

Cancer cell metabolism plays a key role in tumorigenesis and tumor cell proliferation, development, and metastases. In current scenario, cancer metabolism has become a major area of science research, which may provide some information to find new and effective therapeutic approaches to prevent cancer growth (Vernieri et al. 2016). In certain respects, the main metabolic pathways in cancer cells vary in comparison to normal cells, such as their unique capacity to regulate genetics and cellular dynamics, a process known as metabolic rewiring.

Cell metabolism is a biochemical transformation that occurs in all living organisms. Cells are proficient recyclers that break down the complex macromolecules into smaller building blocks leading to the generation of energy units in the form of metabolic currency (ATP) through a process known as catabolism. The anabolic pathway is an energy-consuming process that builds new macromolecules (proteins, lipids, and DNA) to form the cells' biomass. Historically, the only pathways of biosynthesis, such as nucleotides and deoxynucleotides, are intensively studied and targeted fruitfully in cancer therapy (Wilson et al. 2014; Villa et al. 2019).

All living cells depend on metabolic pathways to obtain adequate fuel and metabolic ingredients for the maintenance, proliferation, and critical cell homeostasis. Under normal non-proliferative conditions, cells activate metabolic machines to accommodate ATP intake for reproduction and survival through two oxygen-dependent processes, namely, aerobic respiration and oxidative phosphorylation, where 36 ATP molecules are produced while in anaerobic respiration (Pasteur effect) only 2 ATPs are produced under hypoxic or stressful conditions. Both of these pathways required glucose uptake in order to gain the "energy currency" of the cell.

Classically, healthy or quiescent cells primarily follow aerobic respiration/oxidative phosphorylation to meet their bioenergetic requirements, while cancer cells use

metabolic pathways quite differently from normal cells based on their rapid demand for energy production. To satisfy growth and high proliferation rates, cancer cells prudently adopt various metabolic phenotypes to increase the uptake of nutrients from extracellular environments through the deregulation of metabolic fitness, which is now an emerging hallmark of cancer. The physiological and biochemical aspects of tumor tissues and normal tissues are quite different in many ways.

The majority of these are due to the difference between cancer cell vasculatures and normal cells. Poorly formed tumor vasculature defines a hypoxic microenvironment that results in low levels of nutrients and high levels of waste products (Lugano et al. 2020). Cancer cells respond to such abnormal conditions and adjust their cell metabolism to proliferate and survive even in the worst condition. Aberrant cancer metabolism is one of the silent features of colorectal cancer, which involves the modulation of bioenergetic pathways to meet the rapid demand for bio-molecules to maintain their high rate of growth and energy consumption (DeBerardinis and Chandel 2016).

Colorectal cancer cells are widely known to upregulate in various metabolic pathways, including glycolysis, glutaminolysis, fatty acid biosynthesis, and single-carbon metabolism with oncogenic mutations and loss of tumor suppressor genes (Brown et al. 2018). Overall, cancer cells are switched to increase the rate of aerobic glycolysis despite the presence of an adequate concentration of oxygen and use glucose as a well-known source of energy to accelerate their uncontrolled growth and division. In addition, high pyruvate production by glycolysis is reduced to lactate by fermentation rather than by oxidative phosphorylation.

Primary and metastatic CRCs have been reported to have often shown increased glucose intake and increased intratumoral lactate, as confirmed by tumor imaging with 18F-deoxyglucose positron emission tomography (18FDG-PET) compared to adjacent normal intestinal tissue (Satoh et al. 2017; Maffione et al. 2015). Defective aerobic glycolysis is not effective in producing enough ATP (2 ATP/glucose vs ~36 ATP/glucose by OXPHOS) and it therefore accelerates its nutrient flow by rewiring glycolysis and associated pathways to generate ATP quickly. It can also provide a biosynthetic advantage by supplying precursors and reducing agents for macromolecule synthesis (Burns and Manda 2017).

However, glucose has certain limitations: since it can only supply carbon skeleton for macromolecule biosynthesis, it cannot transport other fuels such as amino acids and glutathione needed by cancer cells during rapid proliferation for nucleotide synthesis and maintenance of oxidative stress. Studies have shown that, after glucose, glutamine is also the most widely used nutrient by cultured cancer cells compared to other non-essential amino acids (Hosios et al. 2016), although abnormal fatty acid metabolism, nucleotides, folate, acetate, and proteins have also been reported (Pavlova and Thompson 2016). Therefore, cancer cells are easily "addicted" to the nutrients, and their withdrawal may eventually result in cell death by apoptosis. Glutamine is a prominent substrate not only for the production of macromolecule biosynthesis (lipids, proteins, and nucleotides) and nicotinamide adenine dinucleotide phosphate (NADPH), but also it serves as an important anaplerotic substrate for TCA or Krebs in proliferating cells (Nguyen and Durán 2018). The difference in metabolism between cancer and normal cells is expected to provide opportunities

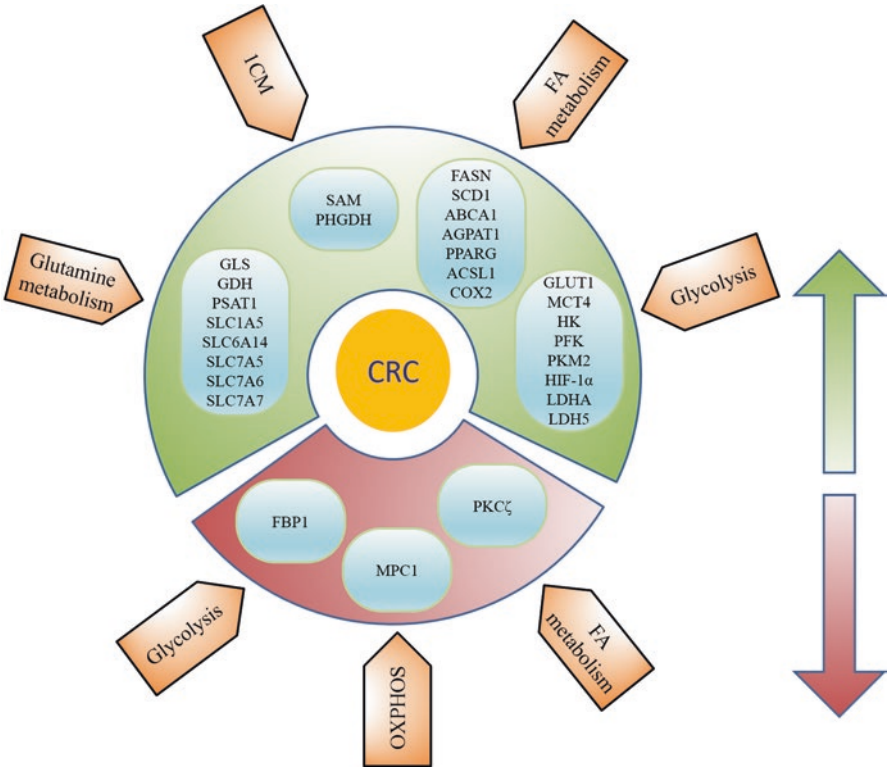

Fig. 15.1 Altered expression of various metabolic processes, enzymes, and transporters associated with the glutaminolysis and connected pathways. Note: Up arrow showing overexpression and down arrow showing downregulation. 1CM one-carbon metabolism

for the development of innovative cancer treatments. Modulated expression of enzymes and transporters involved in various metabolic pathways and nutrient transports in cancer are given in Fig. 15.1.

15.2.1 Glutamine Metabolism

Over the last few decades, oncometabolomics has emerged as a renowned field of interest in cancer biology and has played an important role in targeting various cancers, including colon cancer, and continues to impact our knowledge and understanding of oncology. The addition of new discoveries on cancer metabolism continues to surprise science and opens up additional avenues of research on cancer control. Since the Warburg observation of cancer metabolism, the main focus has been on the fundamental metabolism of carbon, including glycolysis, citric acid cycle, and pentose phosphate pathway.

Current studies have shed light on the role of amino acids in the metabolism of cancer. Amino acids are the main substrate for protein synthesis in cells and tissues. They are vital to the construction and integrity of every cell, without which cells are unable to maintain their morphology and to perform their normal metabolic functions (Vazquez et al. 2016). It can be obtained exogenously and endogenously and has a very significant presence for cell survival, maintenance, and proliferation.

Amino acids also contribute to a wide range of cellular processes, such as energetic regulation, redox homeostasis, nucleotide synthesis, lipids, glucosamines, antioxidants, and polyamines. Hence, this diverse dimension of metabolic activities has made amino acid metabolism increasingly common in cancer research, particularly glutamine metabolism.

Glutamine is the most abundant free amino acid in blood plasma and skeletal muscle (Mayers and Vander Heiden 2015). Glutamine is conventionally a non-essential amino acid, but it has recently been known as a "conditionally essential" nutrient when cells are highly proliferative or have some physiological condition, such as pathogen infection and starvation (Scalise et al. 2020). Further, it is highly versatile amino prudently transferred to its carbon, amino, and amide nitrogen for the generation of other amino acids, nucleotides, and amino sugars. In addition, multiple functions and signaling pathways are involved (Bott et al. 2019).

In cancer cells, glutamine is an alternative source of energy after the glycolytic pathway. Apart from the quiescent cell, cancer cells showed 10–100 times higher glutamine intakes than any others. Higher use of glutamine and subsequent "glutamine dependence" are frequently seen in several cancers, including colorectal cancer, as a result of aberrantly expressed oncogenes and loss of tumor suppressor genes (Miyo et al. 2016; Balsa-Martinez and Puigserver 2015).

As described above, most cancer cells excrete carbon as lactate in the extracellular environment due to deregulated aerobic glycolysis. It makes cancer cells more addicted to glutamine, which not only supplies carbon but also acts as a source of nitrogen for nucleic acid de novo biosynthesis, nicotinamide adenine dinucleotide (NAD), hexosamine, and other non-essential amino acids (NEAAs). It also acts as a precursor to the synthesis of antioxidant enzyme glutathione (GSH) for redox balancing under stressful conditions (Altman et al. 2016).

In contrast, glutamine also induces the absorption of essential amino acids, such as leucine through the plasma membrane, from the extracellular environment via the LAT1/SLC7A5 bidirectional transporter in conjunction with the glutamine efflux. This also stimulates mTOR signaling and helps with unnecessary ammonia and glutamate (GLU) recycling (Scalise et al. 2017). Glutamine participates actively in metabolic rewiring for the replenishment of TCA cycle metabolic intermediates (Hensley et al. 2013). In addition to glutamine, other branched-chain amino acids (BCAAs) such as valine, leucine, and isoleucine can also help promote the growth of cancer cells as an opportunistic fuel for the TCA cycle (Green et al. 2016).

Rapidly proliferating cancer cells make extensive use of glutamine through rewiring associated pathways to accumulate biomass and produce ATP. A tracer finding has shown that up to 50% of NEAAs needed for protein synthesis by cancer cells originate from glutamine, exploring the significant role of glutamine during uncontrolled growth and proliferation (Alberghina and Gaglio 2014).

First, glutamine gives its amide nitrogen to glutamine acid (glutamate) catalyzed by cytoplasmic glutamine (GLS1). GLU is the primary nitrogen donor for other NEAA synthesis. GLU is exchanged for glutamate dehydrogenase (GLUD) with α-ketoglutarate which releases toxic ammonium and further synthesizes lipids and amino acids and gains ATP through the TCA cycle followed by OXPHOS (Li and Le 2018). These biochemical reactions also result in the production of reducing agent NADPH, which is simultaneously used to regenerate glutathione (GSH) which acts as an electron acceptor to counteract reactive oxygen species (ROS) generated in cancer cells during higher metabolic flux (Zhou et al. 2014).

Furthermore, transaminases transfer the rest of nitrogen residues to different α-ketoacids which are glucose or glutamine carbon catabolites by different aminotransferases such as glutamate-pyruvate transaminase (GPT), glutamate-oxalate transaminase (GOT), and phosphoserine aminotransferase 1 (PSAT1) which transfer amino nitrogen from GLU to alanine, aspartate, and phosphoserine, respectively. Aminotransferases also produce α-ketoglutarate without the release of ammonium (Lieu et al. 2020).

Alpha-ketoacid, such as glutamate gamma-semialdehyde, is used to synthesize ornithine that is part of the urea cycle. Serine participates in protein synthesis and also releases some extra carbon from glycolysis into the extracellular environment of cancer cells. Aspartate enters the urea cycle and participates in biosynthesis of asparagine. It also serves as a substrate for purine and pyrimidine synthesis (DeBerardinis and Cheng 2010). Phosphoserine acts as a precursor to glycine and cysteine biosynthesis as part of a single-carbon metabolism. Both amino acids are important for the synthesis of glutathione. In addition, glycine is a key source of carbon and nitrogen in the purine ring (Amelio et al. 2014). Ornithine is a precursor to arginine synthesis. Glutamic acid is donating its carbon and nitrogen to the synthesis of proline. It also produces glutamine via glutamine synthetase (GS) catalysis.

15.2.2 Glutamine Transporters and Cancer

Understanding and targeting cancer cell metabolism has become a well-known task for biochemists and researchers around the world. Transition has been found in the metabolism of glutamine in several different cancers, including colon cancer, which has been observed almost uniformly. As discussed above, glutamine is a vital nutrient known for the plethora of biological functions, including cell growth, proliferation, anaplerosis, redox balance, acid-base balance, and detoxification (NH3) in mammalian cells.

In addition, two supplementary metabolic pathways are also glutamine-dependent: the first glutamine/GABA-glutamine pathway between glutamatergic/GABAergic neurons in the brain and the second glutamine pathway in cancer cells, where glutamine enters the TCA cycle and is subsequently converted to malate and reduced to pyruvate by malic enzyme and then lactate through the action of lactate dehydrogenase. As a result, various glutamine transporters are hired to maintain the concentration of glutamine in blood plasma and cells to perform all of the above-

mentioned functions, including the maintenance of rapid proliferation and cell survival in cancer cells. The blocking of these transporters may also work as a therapeutic weapon in many cancers, including CRC (Cruzat et al. 2018; La Vecchia and Sebastián 2020).

Glutamine is a hydrophilic molecule that cannot simply be diffused into cells across the plasma membrane; therefore, our metabolic system obtains various transporters for transmembrane transfer of glutamine inside the cells. Until recently, 14 glutamine transporters in the plasma membrane have been identified in mammalian cells that recognize and transport glutamine as a substrate (Table 15.1). For humans,

Table 15.1 Compounds currently used to target glutamine metabolism in colorectal cancer

Compounds	Targets	Mechanism of action	In vitro proliferation study in CRC	Preclinical/ clinical study in CRC
CB-839	GLS	Inhibits glutamine metabolism by limiting glutamine uptake through binding with GLS tetramer and targeting mTOR signaling (Sheikh et al. 2017)	Reported (Song et al. 2018)	Phase I clinical trial (Eads et al. 2018)
BPTES			Reported (Song et al. 2017)	—
968		Inhibits the expression of GLS and cancer transformation by disrupting Rho GTPase-dependent mTOR signaling (Tanaka et al. 2015)	Reported (Richard and Martinez 2015)	—
Selenite		Inhibits glutamine uptake via degrading GLS mediated by APC/C-CDH1 pathway (Zhao et al. 2016)	Reported (Zhao et al. 2016)	Tool compound (Zhao et al. 2016)
L-DON	GLS/ GLS2	Inhibits glutamine uptake by disrupting purine biosynthesis and mTOR pathway (Lukey et al. 2013)	Reported (Ahluwalia et al. 1990; Huang et al. 2014)	Phase II clinical trial (Lynch et al. 1982; Mueller et al. 2008)
Acivicin Azaserine				—
L-Asparaginase	ASNS	Inhibits asparagine biosynthesis and mTOR signaling (Toda et al. 2016)	Reported (Toda et al. 2016, 2017)	—
EGCG	GDH	Inhibits GDH by binding to the site of the allosteric regulator ADP (Li et al. 2018)	Reported (Larsen et al. 2010; Jin et al. 2016)	Phase I clinical study (Choi et al. 2018)
AOA	GOT GPT	Impairs protein synthesis by inhibiting pyridoxal phosphate-dependent transaminases (Korangath et al. 2015)	Reported in CRC (Hao et al. 2016)	—

(continued)

Table 15.1 (continued)

Compounds	Targets	Mechanism of action	In vitro proliferation study in CRC	Preclinical/ clinical study in CRC
Benzylserine	SLC1A5	Inhibits glutamine uptake by targeting both SLC1A5 and SLC7A5 and mTOR signaling (Wang et al. 2014)	—	—
GPNA		Binds to SLC1A5 and inhibits glutamine uptake by inducing ROS accumulation (Hassanein et al. 2015)	Reported (Ma et al. 2018)	—
V-9302		Specifically binds to SLC1A5 and blocks glutamine uptake in concentration-dependent manner (Schulte et al. 2018)	Reported (Schulte et al. 2018)	Preclinical tool (Shulte et al. 2018)
Ab3-8		Inhibits the intracellular uptake of glutamine by disrupting AKT-ERK signaling pathway (Hara et al. 2020)	Reported (Hara et al. 2020)	—
BCH	SLC7A5	Inhibits glutamine/leucine exchange and mTOR signaling (Nicklin et al. 2009)	None	—
JPH203		Blocks SLC7A5 by inhibiting mTOR signaling (Häfliger et al. 2018)	Reported (Cormerais et al. 2016; Muto et al. 2018)	Preclinical tool (Oda et al. 2010)
Ab1		Binds to LAT1, thereby impairing transport of LAT1 substrates (Ueda et al. 2019)	Reported (Ueda et al. 2019)	Preclinical tool (Ueda et al. 2019)
Sulfasalazine	SLC7A11	Potentially inhibits SLC7A11 which results in apoptosis (Dixon et al. 2014)	Reported (Ma et al. 2015)	—

glutamine transporters have a 44–55% sequencing identity, and neutral amino acid transporters have a 57% identity. According to the Human Genome Organisation nomenclature, these 14 transporters are distributed among four major gene families, namely, SLC1, SLC6, SLC7, and SLC38. Under normal conditions, glutamine reaches the cells from the extracellular space via the four carrier groups (SLC) of transporters that import glutamine and other amino acids (Scalise et al. 2018). Remember that not all transporters are limited to glutamine and not even just the inflow of glutamine into the cells.

Most of them accept only neutral amino acids, while others accept both neutral and cationic amino acids. Likewise, under normal conditions, most of these transporters are influx extracellular glutamine into the cells and vice versa. Of note, there are a variety of glutamine transporters that are overexpressed in cancer cells to pro-

vide food. Nonetheless, some of the inhibitors against these transporters are currently under review or in a clinical trial. ASCT2 is a Na + upregulated and downregulated transporter of Myc and retinoblastoma protein (Rb), respectively, and belongs to the SLC1 carrier family of genes and facilitates the transport of neutral amino acids, such as glutamine (Reynolds et al. 2014; Gao et al. 2009).

ASCT2 has long been considered a primary high-affinity importer of glutamine to the plasma membrane. SLC1A5 is the only carrier in this family of genes that recognizes glutamine as a substrate (Scalise et al. 2017). In particular, there has been an increased focus on alanine-serine-cysteine transporter 2 (ASCT2) encoded by the SLC1A5 gene, also recognized as an excitatory amino acid transporter (EAAT1-5) due to their elevated expression in various cancers including CRC (Toda et al. 2017; Liu et al. 2018).

The study showed that expression of miRNA-137 altered expression of ASCT2. In addition, it was confirmed that overexpression of ASCT2 was significantly associated with decreased expression of miRNA-137 in CRC (Dong et al. 2017). Recently, a Japanese research group reported that the mutation in the KRAS gene has been significantly associated with ASCT2 expression. They also noticed that the overexpression of the ASCT2 transporter was significantly correlated with tumor depth and vascular invasiveness in *KRAS*-mutant CRC (Toda et al. 2017).

SLC6A14 belongs to the family of SLC6 genes known as Na^+/Cl^--coupled neurotransmitter transporters and recognizes all proteinogenic amino acids except glutamate and aspartate. It is also known as $ATB^{0,+}$ (amino acid transporter with neutral amino acids as a substrate). SLC6A14 is somewhat distinct from SLC1A5, SLC7A5, and SLC7A11 because it is tissue-specific and expressed in the lungs, trachea, salivary gland, pituitary, intestinal tract, and colon. It carrier can transport not only EAAs but also most non-essential amino acids such as glutamine, arginine, serine, and glycine, which are important for the supply of metabolic fuel necessary for tumor development.

SLC7A5 (LAT1) is an Na^+-independent two-way transporter of glutamine, also invlove in transporting BCAAs and neutral amino acids, including EAAs (isoleucine, histidine, methionine, leucine, phenylalanine, tryptophan, tyrosine, valine) (Kandasamy et al. 2018). SLC7A5 is expressed in the colon, fetal liver, intestine, ovary, testis, placenta, spleen, blood-brain barrier, activated lymphocytes, skeletal muscle, heart, lung, thymus, and kidney (Wang and Holst 2015; Pochini et al. 2014). SLC7A5 is an amino acid exchanger that regulates the parallel extracellular efflux of glutamine and the intracellular flow of leucine.

This exchange is regulated by HIF-2α and Myc, which results in the activation of mTORC1 signaling and regulates cell proliferation, differentiation, and growth (Nakazawa et al. 2016). Due to its prominent function in the control of various metabolic enzymes, this transporter has been seen in several cancers such as breast, esophageal, non-small cell lung carcinoma, prostate, biliary, oral, gastric, pancreatic, and colon cancer. Hayase et al. conducted a clinical study and found that the upregulation of the LAT1 transporter was significantly associated with increased cell proliferation in colorectal cancer (Hayase et al. 2017).

Both SLC7A6 and SLC7A7 are neutral/cationic transporters of the SLC7 gene family encoding y + LAT2 and y + LAT1 proteins, of which SLC7A6 is expressed predominantly in the small intestine, parotides, heart, kidney, lung, testis, and brain.

There are unique transport functions such as neutral amino acid inflows and Na^+-dependent efflux. Y + LAT2 was discovered as an arginine/glutamine exchanger in the brain.

Increased expression of SLC7A7 is associated with a high prognosis of several cancers such as malignant glioma and multiple myeloma (Fan et al. 2013) and drug resistance to ovarian cancer and gastric cancer (Cheng et al. 2010; Verma 2020b). Similarly, Lu et al. reported that overexpression of SLC7A6 and SLC7A7 was also essential to the proliferation and development of human colorectal cancer tissue relative to normal colon tissue (Lu et al. 2013). SLC7A8 gene encodes for neutral LAT2 transporter (system L amino acid transporter) where system L is preferred for leucine.

This is identical to SLC7A5 in the selectivity and unique function of the substrate and plays a key role in the discharge of amino acids ingested in the blood from the intestinal and kidney epithelial cells. There is no information as to whether SLC7A8 plays any important role in cancer. Nevertheless, some emerging evidence indicate that many cancers are correlated with high expression of LAT2, but none of them confirm and correlate significantly (Barollo et al. 2016). SLC7A9 is also a Na^+-independent neutral/cationic transporter that is strongly expressed in the stomach, kidney, and placenta. Cationic amino acid is believed to flow through the cells in conjunction with neutral amino acid efflux from the cells. Recently, it has been shown that SLC7A9 is correlated with the proliferation and survival of certain cancers such as breast cancer and papillary thyroid cancer (Jiang et al. 2017; Shen et al. 2018).

Among the 14 glutamine transporters, 6 belong to the SLC38 genes family called SNATs (sodium-coupled neutral amino acid transporters): SNAT1, SNAT2, SNAT3, SNAT5, SNAT7, and SNAT8. Proteins in this gene family transport sodium-coupled neutral, cationic, and anionic amino acids and are considered to be the main transporter of glutamine in human cells. Such transporters are found in intestinal and renal epithelial cells, membranes of the blood vessels, and astrocytes.

Of the six SLC38 gene family, three (SNAT1, SNAT2, and SNAT8) belong to system A-type transporter (Na^+-dependent transportation) common to neutral amino acids like alanine, while SNAT3, SNAT5, and SNAT7 prefer system N transporter (Na^+-dependent transportation) selective for glutamine, asparagine, and histidine; all of these amino acids carry nitrogen atoms in the side chain. SLC38 transporters are commonly recognized for their various biological functions, such as cell proliferation, differentiation, and survival, as they are essential transporters of glutamine (Hägglund et al. 2011).

As a result, the expressions of these transporters are deregulated in many cancers; some studies revealed that SNAT2 could play a role in promoting tumor growth. Some evidence indicates that SNAT2 is a transcriptional target for the TP53 tumor suppressor gene (Grewal et al. 2009). SLC38A1 and SLC38A2 are essential sources of glutamine for glutaminolysis (Bröer et al. 2016). Similarly, elevated levels of SNAT1 in breast and hepatocellular carcinoma were observed (Wang et al. 2013).

Silencing of SLC38A1 also reduced the growth of colon and pancreatic cancer cell lines (Zhou et al. 2017; Xie et al. 2014). It is important to note that SLC38A5 is a transcriptional target for oncogene c-Myc. This transporter is likely to be upregulated in some cancer cells. DeBerardinis et al. reported an increase in the expression of SLC38A5 transporter in Myc-driven cancer cells (DeBerardinis et al. 2007).

In addition, SLC38A3 also plays a vital role in the activation of oncogene due to high reactive oxygen generation in the tumor inflammatory environment (Rubio-Aliaga and Wagner 2016).

15.2.3 Different Enzymes Associated with Glutamine Metabolism in CRC

Glutaminolysis is one of the highly regulated metabolic pathways seen in many cancers. This pathway is widely known for the cancer metabolism due to the conditionally essential amino acid glutamine that the cancer cells are strongly addicted. Various metabolic enzymes are involved in the metabolism of glutamine, which plays many biochemical and physiological roles in normal metabolic signaling.

Glutamine is catabolized by various enzymes, including GLS, carbamoyl-phosphate synthetase 2-aspartate transcarbamylase-dihydroorotase (CAD), or glutamine fructose-6-phosphate amidotransferase (GFAT). Traditionally, glutamine is transported to the cell by two membrane transporters, namely, SLC1A5 and SLC7A5/LAT1, and hydrolyzed to glutamate by GLS/GLS1. In addition, GDH/GLUD1 and aminotransferases convert glutamate to α-KG. Alpha-KG enters the TCA cycle to facilitate the generation of energy in the form of ATP through the production of NADH and FADH2. Glutamine also provides nitrogen and carbon backbone for macromolecules for rapidly proliferating cancer cells.

Mammalian cells encode two genes for glutaminase enzymes: the GLS1 gene is located in chromosome 2 and encodes the kidney-type glutaminase protein (KGA/GLS1) initially characterized in the kidney (Katt et al. 2017; Matés et al. 2013), while the GLS2 gene is found in chromosome 12 and encodes the liver-type protein isoform (LGA/GLS2) originally characterized in the liver. Glutaminase is critically involved in cancer cell proliferation, autophagy, signal transduction, and radioresistance (Sever and Brugge 2015; Xiang et al. 2019).

Glutaminase expression has long been found to be significantly associated with the altered expression of the c-Myc transcription factor and the mTORC1 signaling. The transcription factor c-Myc is a key regulator of the consumption of glutamine and is frequently observed in many proliferating cells, including CRC (La Vecchia and Sebastián 2020). c-Myc also induces the expression of SLC1A5 by promoting the expression of GLS1 and CAD to promote the transport and uptake of glutamine (Bott et al. 2015; Dong et al. 2020).

In addition, retinoblastoma (Rb), a tumor suppressor protein, also negatively regulates the intake of glutamine via the SLC1A5 and GLS1-dependent E2F transcription factor (Csibi et al. 2013). Correspondingly, it has also been reported that the mTOR pathway regulates the expression of GLS by facilitating the translation of c-Myc protein. Similarly, signaling pathways to Rho GTPase can also result in overexpression of NF-kB-dependent GLS1 in cancer cells (Wilson et al. 2013). Huang et al. conducted a case-control study between colorectal tumor and normal tissue and found an increased level of GLS in CRC patients compared to control (Huang et al. 2014).

Li et al. recently reported that the activation of heat shock factor 1 (HSF1) increases the expression of GLS to promote glutamine metabolism and mTOR signaling in colorectal cancer (Li et al. 2018). Unlike GLS/GLS1, the role of GLS2 in cancer metabolism is still controversial and not yet fully understood. Some findings have shown that GLS2 controls the ROS level by targeting the p53 gene by regulating the production of antioxidants (GSH) (Hu et al. 2010; Suzuki et al. 2010). Upregulated expression of GLS2 functions as a tumor suppressor and inhibits the growth and spread of cancer cells. GLS or GDH also plays a pivotal role in the glutamine metabolism by reducing glutamate to α-ketoglutarate through the action of a number of enzymes, including GDH1, GOT2, and GPT2, particularly when the first line of nutrient glucose is insufficient or under hypoxia. In addition, α-ketoglutarate enters the TCA cycle for the production of ATP synthesis and anabolic carbon for other NEAAs, lipids, and nucleotides. Csibi et al. reported that mTORC1 significantly promotes glutaminolysis by activating GDH to promote cell growth, proliferation, and metastases by inhibiting SIRT4 (Csibi et al. 2013).

Similarly, Lorin and coworkers have shown that mTORC1 activation promotes GDH-dependent autophagy by limiting the formation of ROS in cancer cells (Lorin et al. 2013). Some authors have reported overexpression of GDH enzyme in certain types of cancer such as triple-negative breast cancer, lung cancer, glioblastoma, and colorectal cancer (Jin et al. 2016). Wang and his team have recently found that upregulation of SIRT4 directly increases the expression of GLUD1, thus enhancing glutaminolysis in a deglutarylation-dependent manner. In addition, overexpression of SIRT4 has been reported to be significantly associated with poor prognosis of colorectal cancer (Wang et al. 2018).

AST is also known as glutamine oxaloacetate transaminase (GOT1 and GOT2) which is critical for redox homeostasis and growth in some cancer cells that are proliferating. Phosphoserine aminotransferase 1 (PSAT1) catalyzes the transfer of glutamate nitrogen to 3-phosphohydroxypyruvate to make phosphoserine and α-ketoglutarate as part of serine biosynthesis. High expression of PSAT1 has recently been reported to be significantly associated with poor prognosis of colorectal carcinoma (De Marchi et al. 2017).

Similarly, some authors have shown that ALT and AST are particularly associated with the prognosis of certain cancers, such as hepatocellular cancer, renal cell carcinoma, colonic cancer, pancreatic cancer, and breast cancer (Shen et al. 2014; Bezan et al. 2015; Son et al. 2013).

15.3 Targeting Glutamine Metabolism in CRC

Several accumulated data have shown the diverse and significant role of glutamine metabolism in cancer. Since the discovery of "glutamine dependence" in cancer cells, the field has continued to explore and attract many cancer biologists. It is important to develop an effective and gold standard therapeutic agent to target glutamine transporters and enzymes associated with catabolic/anabolic biosynthetic pathways. Until now, few promising compounds target the metabolism of glutamine in certain types of cancer, including colorectal cancer.

Table 15.2 Glutamine transporters for metabolism of glutamine as a main substrate

Gene family	Transporters	Common name	Substrates
SLC1	SLC1A5	ASCT2	Glutamine, alanine, serine, cysteine, asparagine, and threonine
SLC6	SLC6A14	$ATB^{0,+}$	All essential amino acids besides glutamine, lysine, and arginine
	SLC6A19	B^0AT1	Glutamine, leucine, cysteine, valine, isoleucine, methionine, phenylalanine, alanine, serine, and asparagine
SLC7	SLC7A5	LAT1	Glutamine, phenylalanine, tryptophan, tyrosine, valine, leucine, isoleucine, methionine, asparagines, histidine, and threonine
	SLC7A6	y^+LAT2	Glutamine, arginine, and leucine
	SLC7A7	y^+LAT1	Glutamine, arginine, leucine, lysine, and ornithine
	SLC7A8	LAT2	Glutamine, alanine, serine, and glycine
	SLC7A9	$b^{0,+}AT1$	Glutamine, cystine, lysine, and arginine
SLC38	SLC38A1	SNAT1/ ATA1	Glutamine, methionine, proline, serine, asparagine, glycine, alanine, and histidine
	SLC38A2	SNAT2/ ATA2	
	SLC38A3	SNAT3/ SN1	Glutamine, histidine, and asparagine
	SLC38A5	SNAT5/ SN2	Glutamine, alanine, serine, histidine, and asparagine
	SLC38A7	SNAT7	Glutamine, arginine, histidine and asparagine, glutamate, and aspartate
	SLC38A8	SNAT8	Glutamine, asparagine, histidine, alanine, aspartate, and arginine

Some drugs have successfully entered the human clinical trial and/or are approved for use in cancer treatment by the FDA (Table 15.2). The discovery of versatile anti-glutamine compounds is advantageous in cancer therapy. Some small molecule analog glutamine inhibitors, such as acivicin, azaserine, and L-DON, have been tested for their anti-cancer effect in vitro/in vivo, followed by preclinical and clinical studies against multiple types of cancer, including colorectal cancer.

Huang et al. conducted a proliferative study in the colon cancer cell line (HT29, SW480) and found that L-DON significantly reduces growth and proliferation of colon cancer, leading to DNA fragmentation and apoptosis (Huang et al. 2014). In addition to their anti-proliferative role, blocking the metabolism of glutamine in cancer growth has shown significant toxicity which results in adverse effects on the gastrointestinal tract, immune cells, and central nervous system due to non-selective inhibition of the metabolism of glutamine, thus limiting the toxicity of these compounds (Lukey et al. 2013).

GLS inhibition has become much more attractive since the last few years due to the dysregulated expression of GLS in a variety of cancers. In recent years, various GLS inhibitors (BC-839, 968, BPTES) target the metabolism of glutamine by dem-

onstrating tumor suppression in various preclinical tumor models. Of these, CB-839 is considered to be the most advanced and phase I clinical trial compound for many cancers, including colorectal cancer alone and adjuvant therapy (Gross et al. 2014).

Song et al. found that BPTES significantly decreased the growth of colon cancer by inhibiting GLS (Song et al. 2017). Similarly, compound 968 is a GLS1 allosteric regulator that inhibits the growth of Rho GTPase-induced cancer transformation in many cancers (Xie et al. 2016; Verma et al. 2019). Oxaliplatin is an antineoplastic small molecule that is the most commonly used drug for the treatment of colorectal cancer. Some drugs, such as metformin and ribavirin, have the potential to accelerate the synergetic effect of oxaliplatin when combined with compound 968 in colorectal cancer therapy (Richard and Martinez 2015).

Moreover, some studies have revealed the anti-cancer role of EGCG and R162 in colorectal cancer. Furthermore, EGCG and R162 have been shown to significantly inhibit the expression of GDH resulting in reduced tumor aggressiveness and poor prognosis of colorectal cancer (Larsen and Dashwood 2010). Glutamate dehydrogenase is recognized as a novel CRC prognosis marker (Liu et al. 2015), but the clinical significance and importance of GDH expression in colorectal carcinoma has not yet been studied. L-Asparaginase is an antineoplastic enzyme that inhibits asparagine biosynthesis in particular and acts as a depleting agent for plasma asparagine and glutamine in KRAS-mutated colorectal cancer (Altman et al. 2016).

L-Asparaginase is currently the first approved drug choice for the treatment of acute lymphoblastic anemia (ALL) patients (Egler et al. 2016). Benzylserine and GPNA also bind to the SLC1A5 transporter of glutamine and suppress cancer aggressiveness (Hassanein et al. 2015). Ma et al. reported that GPNA-induced inhibition of SLC1A5 sensitizes patients with colorectal cancer resistance to cetuximab (Erbitux) resulting in better therapeutic outcomes in CRC (Ma et al. 2018). In addition, recently published data have shown the role of the novel potent inhibitor V-9302, which inhibits SLC1A5 transporter and colorectal cancer both in vitro and in mice (Schulte et al. 2018).

Hara et al. reported that monoclonal antibody Ab3-8 functions as an antagonist to SLC1A5 and significantly inhibits the growth of KRAS-mutated colon cancer cell line HT29 followed by inhibition of glutamine uptake (Hara et al. 2020). Similarly, anti-human SLC7A5 monoclonal antibody Ab1 is also associated with poor growth and survival of colon cancer (Ueda et al. 2019). Compound JHP20 dramatically blocks the transport of amino acids by SLC7A5 transporter, leading to inhibition of mTOR signaling. It has been shown that JPH203 potentially induced inhibition of leucine uptake by targeting SLC7A5 transporters, which ultimately results in reduced proliferation and cell death in colorectal cancer (Oda et al. 2010).

To date, JPH203 is currently the only SLC7A5 inhibitor to be evaluated in clinical trials for colorectal cancer. A transaminase inhibitor AOA is also associated with poor prognosis and inhibition of colorectal cancer cell growth in vitro and in vivo (Hao et al. 2016). Sulfasalazine (SSZ) is an anti-inflammatory compound that preferably binds to the SLC7A11 cystine transporter and inhibits the growth of colon cancer followed by apoptosis and cell death (Arensman et al. 2019). However, due to its off-target effects in clinical settings, this compound has been limited.

15.4 Future Prospective

Colorectal cancer is one of the most common diseases with high morbidity and mortality affecting millions of lives worldwide. Till now, surgery and chemotherapy are the only treatment options for metastatic CRC that contribute to higher overall survival. However, due to organ-specific toxicity and poor specificity, chemotherapy as a medical procedure is hampered. Over the past few decades, glutamine has been the most regularly tested and studied nutrient in the area of cancer metabolism. Cancer cells have the ability to adopt the metabolic process required for rapid growth and proliferation in order to accommodate the use of nutrients. Glutamine metabolism is a central metabolic pathway that plays a key role in cancer growth and survival regulation by modulating the bioenergetic signal and redox balance by acting as a precursor to biomass accumulation and ATP synthesis.

The key factors that control metabolic reprogramming and make cancer cells more addicted to glutamine are alteration in intrinsic tumor suppressors and oncogenes along with tumor microenvironment. Therefore, some key therapeutic solution may be inserted to target specific cancer metabolism pathways, and a new option may be developed to improve the therapeutic efficiency and specificity. In addition, targeting glutamine metabolism can provide a new roadmap and promising strategy for the pharmacological design of gold standard compounds for the treatment of advanced colorectal cancer and/or chemo/drug resistance. Moreover, anti-glutamine compounds and chemosensitizing drugs as a direct or adjuvant therapy may also work toward improving the quality of life in patients with CRC.

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Chapter 16
Preventive Effect of Indian Food on Colorectal Cancer

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Abstract Cancer, one of the deadliest challenges spreading drastically in the twenty-first century, has now officially become the most dangerous killer in the world according to the World Health Organization. Globally, colorectal cancer (CRC) is among the leading causes of mortality and morbidity throughout the world, thus representing a major public health problem. The modern diet and lifestyle (such as high meat consumption and excessive alcohol use, along with limited physical activity) has led to an increasing mortality rate for colorectal cancer worldwide. Although the incidence rate of CRC in native Indians has been rising slowly over decades, the CRC incidence rate in Central Asia, especially India, is comparatively lower than the Western world. In India the importance of food for healthy well-being is reiterated from a Siddha saying "Unave marunthu, marunthe unavu" meaning food is medicine and medicine is food. The concept in Indian tradition follows a prophylactic route in preventing many diseases. Each and every component of each recipe was well reported for their various biological activities. This chapter provides an extensive insight into Indian approach toward cancer prevention, molecular targets of selected Ayurvedic plants, role of spices and condiments, and dietary phytochemical in colon cancer prevention.

Keywords Colorectal cancer · Ayurveda · Indian diet · Preventive effect

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Abbreviations

5-FU	5-Fluorouracil
ACF	Aberrant crypt foci
A1CF	Apobec-1 complementation factor
ADP ribose	adenosine diphosphate ribose
AGE	Aged garlic extract
AIF	Apoptosis-inducing factor
AKT	Ak strain transforming
AMPK	AMP-activated protein kinase
ASK1	Apoptosis signal-regulating kinase 1
ASR (W)	Age-standardized incidence rates (world)
ATF	Activating transcription factor
Bax	Bcl-2 associated X, apoptosis regulator
Bcl-2	B cell lymphoma 2
BRCA	BReast CAncer gene
CD	Cluster of differentiation
Cdk	Cyclin-dependent kinase
CDX	Caudal type homeobox
CLA	Conjugated linolenic acid
COX	Cyclooxygenase
CRC	Colorectal cancer
CSC	Cancer stem cell
CXCR4	C-X-C chemokine receptor type 4
CYP1A1	Cytochrome P4501A1
DHA	Docosahexaenoic acid
DKK1	Dickkopf-related protein 1
DNA	Deoxyribonucleic acid
DNMT	DNA methyltransferase
EGF	Epidermal growth factor
EGF-R	Epidermal growth factor-receptor
EGR 1	Early growth response protein 1
ElF 2	Eukaryotic initiation factor 2
EPA	Eicosapentaenoic acid
ERCC1	Excision repair cross-complementation group 1
FGF	Fibroblast growth factor
FLT4	Fms-like tyrosine kinase 3
GADD	Growth arrest and DNA damage
GCNT3	Glucosaminyl (N-acetyl) transferase 3, mucin type
GPx	Glutathione peroxidase
GRP	Glucose-regulated protein
GSK3β	Glycogen synthase kinase 3 beta
GSK-3β	Glycogen synthase kinase-3β
HDAC1	Histone deacetylases-1

HIF	Hypoxia-inducible factors
hPXR	Human pregnane X receptor
hTERT	Telomerase reverse transcriptase
IGF 1R	Type 1 insulin-like growth factor receptor
IL	Interleukin
iNOS	Inducible nitric oxide synthase
JNK	c-Jun N-terminal kinase
KIP1	Kinesin-like protein
LC3	Microtubule-associated protein 1A/1B-light chain 3
MAPK	Mitogen-activated protein kinase K
MMP	Matrix metalloproteinase
MOP	Mediator of paramutation
mTOR	Mammalian target of rapamycin
NAG	N-Acetyl beta-D-glucosaminidase
NDRG	N-myc downstream regulated gene
NF-κB	Nuclear factor κB
NMRAL2P	NmrA-like redox sensor 2, pseudogene
NQ01	NAD(P)H quinone dehydrogenase 1
Nrf2	Nuclear factor erythroid 2-related factor 2
OXA	Oxaliplatin
PARP	Poly (ADP-ribose) polymerase
PCNA	Proliferating cell nuclear antigen
PCNA	Proliferating cell nuclear antigen
PGDH	Prostaglandin dehydrogenase
PGE 2	Prostaglandin E2
PGE-2	Prostaglandin E-2
PPAR	Peroxisome proliferator-activated receptors
PRAP	Proline-rich acidic protein
PRP4	Pre-mRNA processing factor 4
PUFAs	Polyunsaturated fatty acids
ROS	Reactive oxygen species
SKP2	S-phase kinase-associated protein 2
SOD	Superoxide dismutase
STAT	Signal transducer and activator of transcription
TCF	Transcription factor
TGF-β	Transforming growth factor-β
TOPO1	Type I topoisomerase
TRAIL	TNF-related apoptosis-inducing ligand
TS mRNA	Thymidylate synthase mRNA
UGT1A	UDP glucuronosyltransferase family 1 member A complex locus
VEGF	Vascular endothelial growth factor
XIAP	X-linked inhibitor of apoptosis protein
ZBTB10	Zinc finger and BTB domain-containing 10

16.1 Introduction

16.1.1 Epidemiology and Etiology of Colorectal Cancer

Cancer, one of the deadliest challenges spreading drastically in the twenty-first century, has now officially become the most dangerous killer in the world according to the World Health Organization. It is a well-known fact that cancer is associated with increasing modernization and advanced pattern of irregular and stressed life dominated by Westernization. Although researchers are making their best efforts to fight this disease, the sure-shot cure is still awaited (Jain et al. 2010).

Globally, colorectal cancer (CRC) is among the leading causes of mortality and morbidity throughout the world, thus representing a major public health problem. It is the third most widespread cancer in both men and women, with more than 690,000 deaths per annum (Ferlay et al. 2015) and the fourth most common cause of oncological death (Torre et al. 2015). In the recent years, the mortality rates of developed countries (e.g., Canada, Australia, the United States, and European countries) are higher than the mortality rates of developing countries (e.g., Africa, Central America, Japan, China, Singapore, and Korea) (Siegel et al. 2016). The modern diet and lifestyle (such as high meat consumption and excessive alcohol use, along with limited physical activity) have led to an increasing mortality rate for colorectal cancer worldwide. The increasing prevalence of obesity and decreasing physical activity in many parts of the world will likely continue to contribute to the growing international colorectal cancer burden if these behaviors are not modified. Although colorectal cancer continues to be a disease of the developed world, incidence rates have been rising in developing countries. Furthermore, the global burden is expected to further increase due to the growth and aging of the population and because of the adoption of Westernized behaviors and lifestyle.

The different stages of colon cancer warrant various treatment options such as chemotherapy, surgery, radiation, and phytotherapy. All other forms of cancer therapeutics have significant adverse effects. As a result, there is a need to develop novel and environmentally benign drug therapies for colorectal cancer.

16.1.2 Global Incidence

There is a wide geographical variation in incidence across the world (Fig. 16.1), with almost 55% of the cases occurring in more developed countries (Ferlay et al. 2013). These geographic differences may be attributable to different dietary and environmental exposures that are imposed upon a background of genetically determined susceptibility. Countries with the highest incidence rates include Australia, New Zealand, Europe, and Northern America. Conversely, incidence rates are low in Africa, South-Central Asia, and Central America. Globally, incidence rates vary tenfold, the highest estimated rates being in Australia/New Zealand (age-standardized ratio 44.8 and 32.2 per 100,000 in men and women, respectively) and the lowest in Western Africa (4.5 and 3.8 per 100,000).

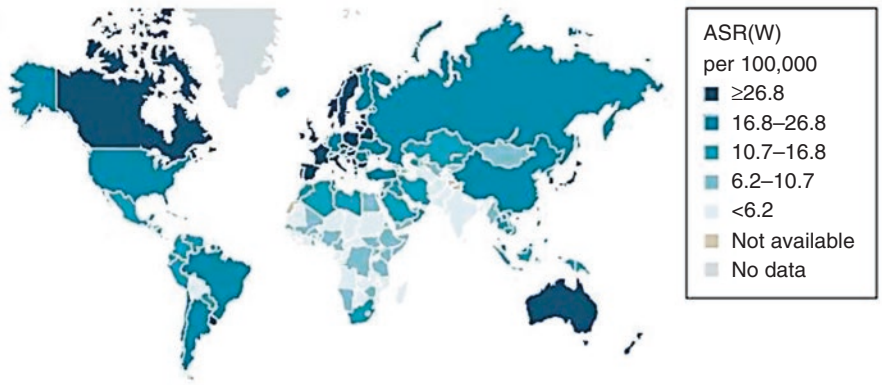

Fig. 16.1 Global incidence of CRC. (Source: Keum and Giovannucci 2019; https://www.nature.com)

On the other hand, CRC incidence rates have been rising in developing countries (Jemal et al. 2010). The greatest increases have been observed in Western Asia (Israel) and Eastern Europe (Czech Republic, Slovakia, and Slovenia). This rise may reflect an increased prevalence of risk factors for CRC that are associated with Westernization such as unhealthy diet, obesity, and smoking prevalence (Center et al. 2009; Martin et al. 2008). Low socioeconomic status is associated with an increased risk for CRC. This may be due to a higher incidence of potentially modifiable risk factors (physical inactivity, unhealthy diet, smoking, obesity) as well as low rates of CRC screening (Doubeni et al. 2012).

Incidence rates are substantially higher in men than in women in most parts of the world. The reasons for this variability are not completely understood but likely reflect complex interactions between sex-specific exposure to risk factors and protective effects of both endogenous and exogenous hormones as well as gender-specific differences in screening practices (Murphy et al. 2011; Meissner et al. 2006). CRC incidence rates also rise with increasing age (Singh et al. 2014). CRC is uncommon among people aged 40 or younger; the incidence begins to raise significantly between the ages of 40 and 50, and age-specific incidence rates further increase in each succeeding decade thereafter.

16.1.3 Incidence Rate in India

Although the incidence rate of CRC (Table 16.1) in native Indians has been rising slowly over decades and the incidence of CRC in immigrant Indians living in the United Kingdom and the United States has been rising rapidly, the CRC incidence rate in Central Asia, especially India, is comparatively lower than the Western world.

Table 16.1 Incidence rate of CRC in India

Country (incidence rate per 100,000)	Male	Female
India	4.3	3.4
Yemen	4.3	4.6
Bangladesh	4.5	4.0
Egypt	4.6	4.3
Pakistan	4.9	4.2
Iraq	5.2	4.0
Nepal	5.3	4.8
Syrian Arab Republic	6.5	4.9
Afghanistan	6.9	7.0
Oman	7.3	6.8
United Arab Emirates	7.3	8.4
Sri Lanka	7.5	5.8
Bhutan	7.9	4.4
Islamic Republic of Iran	8.7	6.4
Qatar	10.5	11.8
Kuwait	11.8	13.4
Myanmar	12.2	12.0
Bahrain	12.4	7.8
Turkey	13.2	9.1
Saudi Arabia	14.3	9.8
Jordan	15.2	11.9
China	16.3	12.2
Indonesia	19.1	15.6
Malaysia	19.6	15.5
Russian Federation	29.0	21.2
United States of America	34.1	25.0
United Kingdom	36.2	23.5
Singapore	41.6	28.3
Japan	41.7	22.8
Israel	41.7	22.8
Republic of Korea	46.9	25.8

Finding the factors responsible for the low incidence of CRC in India will help in primary prevention of CRC. For decades it has been believed that the predominantly vegetarian diet with high fiber and low meat intake is responsible for the low CRC incidence in India. All the large prospective studies and meta-analysis suggest that other causative possibilities need to be examined. Plausible mechanisms include consumption of fewer calories and alcohol by vegetarians as well as diet-induced variations in the intestinal immunity modulated by the gut microbiota. In the absence of robust data from controlled clinical trials, the general principles for cancer prevention should have a holistic approach with emphasis on a healthy diet and health promotion activities.

Nowadays, there has been a rapidly increasing problem of vitamin D deficiency among rural and urban Indians. Meta-analysis of several studies confirms the protective role of physical activity in CRC prevention in both men and women (Wolin et al. 2009). Consumption of moderate amounts of fruit and vegetables sufficient to prevent micronutrient deficiencies, avoiding excess weight gain by balancing energy intake and energy expenditure with physical activity, and avoiding tobacco in all forms will remain the ultimate means to prevent several cancers and contribute to health gains in our community.

16.1.4 Mortality and Survival

There is less variability in mortality rates (Fig. 16.2) worldwide (sixfold in men, fivefold in women), with the highest mortality rates in both sexes estimated in Central and Eastern Europe (20.3 per 100,000 for males, 12.1 per 100,000 for females) and the lowest in Middle Africa (3.5 and 2.7, respectively) (Ferlay et al. 2013). Mortality varies substantially by sex and race. Rates are 30–40% higher in men than in women overall (Siegel et al. 2014).

As for both incidence and mortality, survival rates vary by race/ethnicity (Jackson-Thompson et al. 2006). Most of the marked global and regional disparity in survival is likely due to differences in access to diagnostic and treatment services. Large differences in survival rate according to the stage of disease at diagnosis are observed worldwide (Jemal et al. 2004).

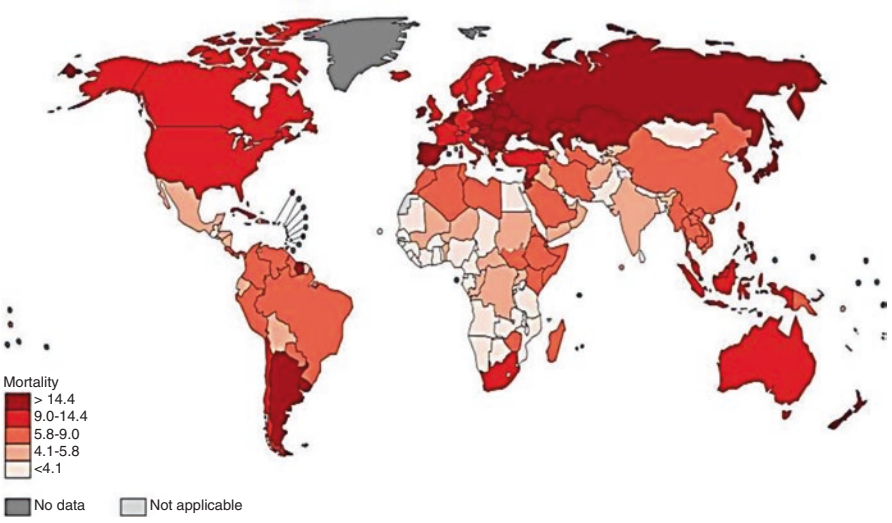

Fig. 16.2 Global morality rate for CRC. (Source: Melina Arnold et al. 2016)

16.2 Indian Approach Toward Cancer Prevention

Ayurveda (which means the science of long life), the oldest Indian indigenous medicine system of plant-based drugs, is known from very early times (at least 5000 years old) for preventing or suppressing various tumors using natural compounds derived from herbs. Nowadays, scientists are yearning toward complementary approach and alternative medicine for the management of cancer as the modern cancer therapy is known to produce drug-induced toxic side effects although it hopes for perfect cure of the disease. The commonly used herbal decoctions reported in Ayurveda are made of multiple herbs possessing a great potential for a cancer cure. Indian medicinal plants are listed in Table 16.2. Scientifically these formulations work on multiple biochemical pathways and influence different organ systems all together and nourish the body as a whole by supporting body's defense systems. Herbs help in complete healing and reduce side effects and cancer-associated complications (Smit et al. 1995).

There are several common features between the Ayurvedic concept of cancer and those currently practiced. These include surgery followed with treatment with drugs derived from plants. Cancer medicine currently practiced is meant to inactivate or activate specific molecules or cell signaling pathways. Within the last three decades, cancer-causing genes called oncogenes, cancer-suppressing genes (tumor-suppressor genes), cancer growth factors (such as epidermal growth factor and vascular endothelial growth factor), cancer-promoting enzymes (such as cyclooxygenase [COX]-2, matrix metalloproteinase 9, inducible nitric oxide synthase), and cancer-causing protein kinases (AKT, mitogen-activated protein kinase [MAPK], protein kinase C) have been identified as targets. Although these targets were not known 5000 years ago, the components of herbs used at that time now appear to target these molecules (Table 16.3). For instance, nuclear factor kB, which has been known to play a major role in tumorigenesis, is targeted by the components of several herbal remedies described in Ayurveda (24–70). Similarly, several herbs have been described in Ayurveda that can suppress either expression of COX-2 or its activity (Takada et al. 2005, 2006; Ichikawa et al. 2006; Sandur et al. 2006; Fukuda et al. 1999; Hong et al. 2002; Kiemer et al. 2003; Li et al. 2004; Chun et al. 2003; Androulakis et al. 2006; Huss et al. 2002).

Development of new synergistic anticancer agents based on these herbs would be beneficial for modern treatment modalities. The use of *Vinca rosea* in the treatment of cancer is very well described in Ayurveda. Modern medicine has shown that vincristine, derived from the plant *Vinca rosea*, can be used as a standard therapy for several cancers.

Nowadays, many herbs are under clinical studies to investigate their phytochemical and antioxidant properties in order to understand their anticancer potential. More than 25% of the drugs used in the last 20 years are directly derived from plants, while the other 25% are chemically altered natural products. Nine plant-derived compounds including vinblastine, vincristine, etoposide, teniposide, taxol, navelbine, taxotere, topotecan, and irinotecan have been approved for use as

Table 16.2 Indian medicinal plants

S. No.	Scientific name	Common names (English)	Indian names (Sanskrit)	References
1.	*Abrus precatorius*	Jequirity bean, rosary pea, Indian licorice	Gunja	Garodia et al. (2007); https://en.wikipedia.org; http://www.flowersofindia.net
2.	*Aegle marmelos*	Bilwa, bael, stone apple, Bengal quince, golden apple, stone apple, or wood apple	Adhararuha, Sivadrumah	Garodia et al. (2007); https://ayurwiki.org
3.	*Albizia lebbeck*	Lebbek tree, flea tree, frywood, koko, woman's tongue tree	Sirisha	Garodia et al. (2007); https://en.wikipedia.org; http://www.flowersofindia.net
4.	*Allium sativum*	Garlic	Lasuna, Ugragandha, Bhootaghni, Rasona	http://www.ayurvedavignan.com; Garodia et al. (2007); https://en.wikipedia.org
5.	*Aloe vera*	Aloe, true aloe	Ghrit Kumari	https://www.indiastudychannel.com; Garodia et al. (2007); https://en.wikipedia.org
6.	*Alstonia scholaris*	Blackboard tree or devil's tree	Saptacchada, Saptaparni, Saptaahva	Garodia et al. (2007); https://ayurwiki.org
7.	*Anacardium occidentale*	Cashew	Agnikrita, Arushkara, Guchhapushpa, Kajutah, Kajutaka, Vrkkaphalah	Garodia et al. (2007); https://ayurwiki.org
8.	*Annona squamosa*	Custard apple, sugar apple, sweetsop	Agrimakhya, atripya, bahubijaka, ganda, gandagatra, gandhagatra, gutea, krishnabija, krisnabija, shubha, sitapalam, sitaphala, subha, suda, vaidehivallabha	http://envis.frlht.org; Garodia et al. (2007); https://ayurwiki.org; https://en.wikipedia.org; http://www.flowersofindia.net
9.	*Aristolochia indica*	Indian birthwort, Duck flower	Arkamula, isvari, rudrajata, sunanda, vishapaha	http://envis.frlht.org; Garodia et al. (2007); https://ayurwiki.org; https://en.wikipedia.org; http://www.flowersofindia.net
10.	*Asparagus racemosus*	Buttermilk root, climbing asparagus, water root, wild asparagus, wild carrot	Shatavari, Satavar, Satmuli	http://envis.frlht.org; Garodia et al. (2007); https://ayurwiki.org; https://en.wikipedia.org; http://www.flowersofindia.net

(continued)

Table 16.2 (continued)

S. No.	Scientific name	Common names (English)	Indian names (Sanskrit)	References
11.	*Azadirachta indica*	Neem, nim tree or Indian lilac, Margosa tree, Indian cedar, paradise tree	Pakvakrita, nimbaka, Nimba, Nim, Nimb	http://envis.frlht.org; Garodia et al. (2007); https://ayurwiki.org; https://en.wikipedia.org; http://www.flowersofindia.net; http://herbfinder.himalayawellness.in
12.	*Bacopa monnieri*	Indian pennywort, herb of grace	Brahmi, Tiktalonika	Garodia et al. (2007); https://ayurwiki.org
13.	*Bauhinia racemosa*	Burmese silk orchid, Bidi leaf tree	Yamalapatrakah, Yugmapatra	http://envis.frlht.org; Garodia et al. (2007); https://ayurwiki.org; https://en.wikipedia.org; http://www.flowersofindia.net; http://herbfinder.himalayawellness.in
14.	*Berberis aristata*	Indian barberry, "chutro," or tree turmeric	Daruharidra, Darvi	https://en.wikipedia.org; http://www.flowersofindia.net; Garodia et al. (2007)
15.	*Bergenia ligulata*	Velvet leaf	Ashmabheda, Nagbhita, Asmabhedaka, Nagbhita	Garodia et al. (2007); https://ayurwiki.org
16.	*Boswellia serrata*	Indian frankincense tree, Indian olibanum	Shallaki	Garodia et al. 2007; https://en.wikipedia.org; https://ayurwiki.org; https://www.planetayurveda.com
17.	*Brassica campestris*	Field mustard	Raktasarshapa	https://www.indianmedicinalplants.info; Garodia et al. (2007); https://ayushvedah.com
18.	*Brassica oleracea*	Cabbages, cauliflower	Dalamalani, dalasarini, kebuka, kechuka, keluta, kembu, kembukah, kemuka, pechuka, pichuni, polini, supatra, swadukanda, swalpavitapa	http://envis.frlht.org; Garodia et al. (2007); https://en.wikipedia.org
19.	*Calotropis gigantea*	Crown flower or giant milkweed	Alarkah, arka	https://www.easyayurveda.com; http://envis.frlht.org; Garodia et al. (2007); https://en.wikipedia.org
20.	*Cassia angustifolia*	Indian senna, Tinnevelly senna	Swarnapatri	https://www.easyayurveda.com; https://livayur.com; http://envis.frlht.org; Garodia et al. (2007); https://en.wikipedia.org

S. No.	Scientific name	Common names (English)	Indian names (Sanskrit)	References
21.	*Cedrus deodara*	Deodar cedar, Himalayan cedar	Devdar	https://en.wikipedia.org; http://www.flowersofindia.net; http://envis.frlht.org; Garodia et al. (2007)
22.	*Centella asiatica*	Indian pennywort or Asiatic pennywort	Aindri, bheka-parni, bhekaparnika, bhandi, brahmamanduki, brahmi, mandukaparni, manduki, sarasvati, bhekaparni, bheki, cheka-parni, darduchhada, divya, mahaushadhi, mandukaparnika, supriya, tvashti	https://en.wikipedia.org; http://www.flowersofindia.net; http://envis.frlht.org; Garodia et al. (2007)
23.	*Cinnamomum cassia*	Bastard cinnamon	Coca, gudatvac, gudatvak, thracham, thwak, tvak, twak, varanga, varangam	https://en.wikipedia.org; http://envis.frlht.org; Garodia et al. (2007)
24.	*Citrullus colocynthis*	Bitter apple, bitter cucumber, desert gourd, egusi, vine of Sodom, or wild gourd	Aindri, Atmaraksha, Brihadvaruni, Brihatphala, Chitrala, Chitrapala, Indravaruni, Mrgabojani, Mahendravaruni	https://www.easyayurveda.com; http://envis.frlht.org; Garodia et al. (2007); https://en.wikipedia.org
25.	*Commiphora mukul*	Indian bdellium	Guggulu	https://www.dabur.com; http://envis.frlht.org; http://herbfinder.himalayawellness.in; https://www.wisdomlib.org; Garodia et al. (2007)
26.	*Coptis teeta*	Indian goldthread	Mamira, Tiktamula	http://envis.frlht.org; https://www.ayurtimes.com; Garodia et al. (2007)
27.	*Coriandrum sativum*	Coriander	Vedhaka, Veshana, Vitunnaka, Dhanyaka	http://envis.frlht.org; Garodia et al. (2007); https://ayurwiki.org
28.	*Curcuma longa*	Turmeric	Haridra, Rajani, Nisha Haldi, Halada	http://envis.frlht.org; Garodia et al. (2007); http://www.flowersofindia
29.	*Curcuma zedoaria*	Zedoary, white turmeric, or temu putih	Dravida, durlabha, gandhamula, gandhamulaka, gandhasara, jatala, kaccura, kachhuraha, kachura, kacoraka, kalpaka, karchura, karcura, karcurah, karshya, krachura, mukhya, nirvisha, sathi, sati, shathi, shati, vanaharidra, vedhya	https://www.biodiversityofindia.org; Garodia et al. (2007)

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Table 16.2 (continued)

S. No.	Scientific name	Common names (English)	Indian names (Sanskrit)	References
30.	*Cydonia oblonga*	Common quince	Amrutaphala	http://envis.frlht.org; Garodia et al. (2007)
31.	*Cymbopogon citratus*	Lemongrass	Aaavartaki	Garodia et al. (2007); https://ayurwiki.org; http://www.flowersofindia; http://envis.frlht.org
32.	*Cymbopogon martinii*	Palmarosa Indian geranium, ginger grass, rosha, and rosha grass	Dhyamakah, Rohisa, Rohisah	https://www.easyayurveda.com; http://envis.frlht.org; Garodia et al. (2007)
33.	*Datura metel*	Angel's trumpet	Datura	Garodia et al. (2007); https://ayurwiki.org
34.	*Euphorbia hirta*	Hairy spurge	Dugadhika	http://www.instituteofayurveda.org; Garodia et al. (2007)
35.	*Foeniculum vulgare*	Fennel	Satupusa	Garodia et al. (2007); https://ayurwiki.org
36.	*Glycine max*	Soybean, soya	Soyabean	https://herbsandayurveda.wordpress.com; Garodia et al. (2007)
37.	*Glycyrrhiza glabra*	Liquorice	Yastimadhu	Garodia et al. (2007); https://newdrugapprovals.org
38.	*Gmelina arborea*	Beechwood, gmelina, goomar teak, Kashmir tree, Malay beechwood, white teak, yamane	Gambhari, Bhadraparni, Shriparni, Madhuparnika, Kasmiri, Kashmari, Hora, Kashmarya, Pitharohini, Madhu rasa	https://www.planetayurveda.com; Garodia et al. (2007)
39.	*Heliotropium indicum*	White clary	Hastishundi, Shrihastini, Vruschikali	http://www.instituteofayurveda.org; Garodia et al. (2007)
40.	*Holarrhena antidysenterica*	Kurchi tree	Vatsak	Garodia et al. (2007); http://envis.frlht.org
41.	*Indigofera tinctoria*	Indigo	Anjanakesika, Asita	Garodia et al. (2007); https://ayurwiki.org
42.	*Inula cappa*	Sheep's ear	Poushkara puskaramula sugandhikam	Garodia et al. (2007); https://www.planetayurveda.com; http://www.flowersofindia

S. No.	Scientific name	Common names (English)	Indian names (Sanskrit)	References
43.	*Jasminum auriculatum*	Jasmine	Yuthika	Garodia et al. (2007); http://www.flowersofindia; https://www.easyayurveda.com; http://envis.frlht.org
44.	*Juniperus communis*	Juniper	Aparajita, ashvathaphala, dhmankshanashini, habusha, hapusa, hapusha, havusa, kachhughna, kaphaghni, kapotapanka, matsyagandha, plihahantri, plihashatru, svalpaphala, vapusha, vigandhika, vishaghni, visra, visraganga	Garodia et al. (2007); https://www.easyayurveda.com; http://envis.frlht.org
45.	*Leea macrophylla*	Dinda Hathikana	Dhola samudrika, dholasamudrika, hastikanda, hastikarnapalasha, hastiparni, kekidanda, morata, samudrak	Garodia et al. (2007); https://www.easyayurveda.com; http://envis.frlht.org; http://www.flowersofindia
46.	*Luffa cylindrica*	Dishrag gourd	Dhamargava mahakosataki, rajakausataki, rajakosataki, sodhani, aibhi, brihatkoshataki, dirgha-patola, dirghapatolika, ghoshaka, hastighosha, hastikoshataki	Garodia et al. (2007); https://www.easyayurveda.com
47.	*Mallotus philippensis*	Kamala tree	Kampillaka	Garodia et al. (2007); https://www.easyayurveda.com
48.	*Manilkara hexandra*	Khirni	Kshirini	Garodia et al. (2007); http://www.flowersofindia
49.	*Medicago sativa*	Alfalfa, lucerne	Vilaayatigawuth, Lasunghaas	Garodia et al. (2007); http://envis.frlht.org
50.	*Melia azedarach*	Chinaberry tree, Pride of India, bead tree, Cape lilac, syringa berry tree, Persian lilac, Indian lilac, or white cedar	Mahanimba	Garodia et al. (2007); https://www.bimbima.com
51.	*Moringa oleifera*	Horseradish tree	Akshiba, aksiva bahala-pallavah, bahalah, bahumula, chaksushya, etc.	Garodia et al. (2007); http://envis.frlht.org

(continued)

Table 16.2 (continued)

S. No.	Scientific name	Common names (English)	Indian names (Sanskrit)	References
52.	*Nerium indicum*	Oleander	Hayamaaraka, Harapriya	Garodia et al. (2007); https://ayurwiki.org
53.	*Nigella sativa*	Black cumin	Kalonji, Kalajira, Kalajaji, Mugrela, Upakuncika	Garodia et al. (2007); http://envis.frlht.org; https://www.easyayurveda.com
54.	*Ocimum sanctum*	Holy basil	Tulsi	Garodia et al. (2007); http://envis.frlht.org; https://www.planetayurveda.com
55.	*Phyllanthus amarus*	Carry Me Seed, Black catnip, child pick-a-back	Bahupatra, Bhumyamalaki	Garodia et al. (2007); http://www.flowersofindia.net; https://ayurwiki.org
56.	*Phyllanthus fraternus*	Leaf flower	Tamalaki	Garodia et al. (2007); https://www.flowersofindia.net
57.	*Picrorhiza kurroa*	Kutki	Anjani, arishta, Katumbhara	Garodia et al. (2007); https://www.flowersofindia.net
58.	*Piper betle*	Betel leaf pepper	Tambula, Saptashira	Garodia et al. (2007); https://www.flowersofindia.net; https://ayurwiki.org; http://envis.frlht.org
59.	*Pisum sativum*	Garden pea	Satila, tripua, renuka	Garodia et al. (2007); http://envis.frlht.org
60.	*Plumbago rosea*	Radix plumbago	Citraka, dahana	Garodia et al. (2007); http://envis.frlht.org
61.	*Plumbago zeylanica*	Leadwort	Anala, pithi, jyothi, vahni, sikhi	Garodia et al. (2007); http://envis.frlht.org; https://www.planetayurveda.com
62.	*Punica granatum*	Pomegranate	Dadima	Garodia et al. (2007); http://envis.frlht.org; https://herbs.indianmedicinalplants.info
63.	*Rubia cordifolia*	Indian madder	Manjistha	Garodia et al. (2007); https://www.flowersofindia.net
64.	*Rumex crispus*	Yellow dock	Amla-vedasa, shula-vedhi-chukra	Garodia et al. (2007); http://envis.frlht.org
65.	*Salvia officinalis*	Sage	Samudraphala, shati, vrddhadaru	Garodia et al. (2007); https://www.flowersofindia.net
66.	*Saraca indica*	Ashoka tree	Ashoka, Gandhapushpa	Garodia et al. (2007); https://www.easyayurveda.com

S. No.	Scientific name	Common names (English)	Indian names (Sanskrit)	References
67.	*Saussurea lappa*	Indian costus root, kuth, kustha	Aalaya, Paakala	Garodia et al. 2007; https://www.flowersofindia.net; http://envis.frlht.org;
68.	*Semecarpus anacardium*	Marking nut	Ahvala, arshastah, arudhkh, bhallatakah, vahnih, vishasya	Garodia et al. (2007); http://www.flowersofindia.net
69.	*Syzygium cumini*	Jambolan, Java plum, jamun	Brahaspati, jambavam, jambu, jambuh, jambula, kakajambu, mahajambu, mahaskandha, meghamodini, meghavarna, nilaphala, nilaprala, rajajambu, rajaphala, rajasha, rajphala, shukapriya, shyamala, surabhipriya, svetajambu, jambava, mahaphala, phalendra, raja-jambuh	Garodia et al. (2007); http://envis.frlht.org
70.	*Tribulus terrestris*	Caltrops, puncture vine	Goksur, kshurk, trikant, swadukantak	Garodia et al. (2007); https://www.easyayurveda.com
71.	*Tylophora asthmatica*	Indian ipecac, emetic swallowwort, Indian ipecacuanha	Lataksiri	Garodia et al. (2007); http://iu.ff.cuni.cz
72.	*Vernonia cinerea*	Ash-colored fleabane, purple fleabane	Sahadevi, ardhaprasadana, dandotpala, devasasha, devika, gandhavalli, govandani, saha, sahadeva, vishamajvaranashini, vishvadeva	Garodia et al. (2007); http://envis.frlht.org
73.	*Vinca rosea*	Periwinkle	Nityakalyani, sadapushpi, rasna, sadampuspa	Garodia et al. (2007); http://envis.frlht.org
74.	*Vitis vinifera*	Grape	Draksha	Garodia et al. (2007); http://envis.frlht.org
75.	*Withania somnifera*	Indian ginseng	Ashwagandha	Garodia et al. (2007); http://envis.frlht.org; http://www.flowersofindia.net; https://herbs.indianmedicinalplants.info; https://www.planetayurveda.com
76.	*Zingiber zerumbet*	Shampoo ginger, bitter ginger	Ahava, Avanti, karpuraharidra, kolanjana, kumbhika, sthulagranthi, viranam	Garodia et al. (2007); https://www.flowersofindia.net; http://envis.frlht.org

Table 16.3 Molecular targets of selected Ayurvedic plants (Garodia et al. 2007)

Molecular target		*Herbs*
Transcription factors	Nuclear factor κB (NF-κB)	*Curcuma longa*, *Withania somnifera*, *Boswellia serrata*, *Aloe vera*, *Allium sativum*, *Saussurea lappa*, *Ocimum sanctum*, *Plumbago zeylanica*, *Brassica oleracea*, *Semecarpus anacardium*, *Phyllanthus amarus*, *Rumex crispus*, *Cydonia oblonga*, *Punica granatum*, *Coriandrum sativum*, *Vitis vinifera*, *Gmelina arborea*, *Commiphora mukul*, *Juniperus communis*, *Citrullus colocynthis*, *Syzygium cumini*, *Brassica campestris*, *Indigofera tinctoria*, *Bergenia ligulata*, *Dysoxylum binectariferum*, *Boswellia serrata*, *Salvia officinalis*, *Foeniculum vulgare*, *Cassia angustifolia*, *Glycine max*, *Tanacetum parthenium*, *Zingiber zerumbet*
	Signal transducer and activator of transcription (STAT)-3	*Curcuma longa*, *Citrullus colocynthis*, *Indigofera tinctoria*
	Nrf2	*Curcuma longa*, *Vitis vinifera*
Growth factors	Epidermal growth factor (EGF)	*Curcuma longa*
	Transforming growth factor-β (TGF-β)	*Curcuma longa*
	Vascular endothelial growth factor (VEGF)	*Curcuma longa*, *Boswellia serrata*, *Commiphora mukul*, *Indigofera tinctoria*, *Plumbago zeylanica*, *Vitis vinifera*, *Gmelina arborea*
	Her2/neu	*Aloe vera*, *Rumex crispus*
Receptors	Androgen receptor	*Curcuma longa*, *Aloe vera*, *Vitis vinifera*
	EGF-R	*Curcuma longa*
	Estrogen receptor-α	*Curcuma longa*
	Fas-R	*Curcuma longa*
Invasion/ metastasis	Matrix metalloproteinases	*Curcuma longa*, *Boswellia serrata*, *Aloe vera*, *Plumbago zeylanica*, *Rumex crispus*, *Gmelina arborea*, *Commiphora mukul*, *Indigofera tinctoria*, *Dysoxylum binectariferum*, *Salvia officinalis*, *Zingiber zerumbet*
	Inducible nitric oxide synthase	*Curcuma longa*, *Phyllanthus amarus*, *Cydonia oblonga*, *Vitis vinifera*, *Tribulus terrestris*
	Nitric oxide	*Saussurea lappa*, *Boswellia serrata*, *Nigella sativa*, *Aegle marmelos*, *Cydonia oblonga*
	Cyclooxygenase-2	*Curcuma longa*, *Withania somnifera*, *Boswellia serrata*, *Plumbago zeylanica*, *Phyllanthus amarus*, *Vitis vinifera*, *Coptis teeta*, *Tribulus terrestris*, *Tinospora smilacina*, *Commiphora mukul*, *Indigofera tinctoria*, *Salvia officinalis*, *Zingiber zerumbet*, *Nigella sativa*, *Cinnamomum cassia*, *Curcuma zedoaria*

(continued)

Table 16.3 (continued)

Molecular target		*Herbs*
Inflammatory cytokines	Tumor necrosis factor-α	*Curcuma longa*, *Saussurea lappa*, *Curcuma zedoaria*
	Interferon-ι	*Cydonia oblonga*
	Interleukin (IL)-1	*Curcuma longa*, *Saussurea lappa*, *Phyllanthus amarus*, *Vitis vinifera*
	IL-4	*Gmelina arborea*, *Medicago sativa*, *Curcuma zedoary*, *Indigofera tinctoria*
	IL-6	*Curcuma longa*, *Vitis vinifera*
	IL-8	*Curcuma longa*, *Saussurea lappa*, *Vitis vinifera*
Protein kinase	Extracellular signal-regulated kinase	*Curcuma longa*, *Boswellia serrata*, *Saussurea lappa*, *Rumex crispus*, *Cydonia oblonga*, *Vitis vinifera*, *Cassia angustifolia*
	c-Jun N-terminal kinase (JNK)	*Curcuma longa*, *Boswellia serrata*, *Saussurea lappa*, *Coriandrum sativum*, *Vitis vinifera*
	Mitogen-activated protein kinase (MAPK)	*Curcuma longa*, *Boswellia serrata*, *Saussurea lappa*, *Coriandrum sativum*, *Foeniculum vulgare*
	Protein kinase C	*Curcuma longa*, *Vitis vinifera*
	AKT	*Curcuma longa*, *Gmelina arborea*, *Indigofera tinctoria*
Enzymes	Adenosine triphosphatase	*Curcuma longa*, *Alstonia scholaris*
	Glutathione S-transferase	*Curcuma longa*
Apoptosis	Bcl-2	*Curcuma longa*, *Boswellia serrata*, *Plumbago zeylanica*, *Brassica oleracea*, *Vitis vinifera*, *Gmelina arborea*, *Commiphora mukul*, *Brassica campestris*, *Indigofera tinctoria*, *Zingiber zerumbet*
	Bcl-xl	*Curcuma longa*, *Boswellia serrata*, *Plumbago zeylanica*, *Brassica oleracea*, *Vitis vinifera*, *Brassica campestris*
	Bax	*Vitis vinifera*
	Survivin	*Plumbago zeylanica*, *Vitis vinifera*
	Caspases	*Aloe vera*, *Cymbopogon winterinus*, *Cymbopogon martinii*, *Vitis vinifera*, *Cymbopogon citrus*
Cell cycle	p53	*Curcuma longa*, *Vitis vinifera*
	p21 $^{Cip1/WAF1}$	*Vitis vinifera*, *Gmelina arborea*, *Glycyrrhiza glabra*, *Indigofera tinctoria*
	Cyclin D1	*Curcuma longa*, *Boswellia serrata*, *Plumbago zeylanica*, *Vitis vinifera*, *Commiphora mukul*, *Indigofera tinctoria*, *Dysoxylum binectariferum*, *Salvia officinalis*, *Zingiber zerumbet*, *Vitis vinifera*, *Gmelina arborea*

anticancer drugs (Jain et al. 2010). 10-Hydroxycamptothecin, monocrotaline, d-tetrandrine, lycobetaine, indirubin, colchicinamide, curcumol, curdione, gossypol, and homoharringtonine are a few more plant-derived compounds of high hope (Cravotto et al. 2010; Patel et al. 2010). Each herb contains multiple active principles that often operate synergistically producing therapeutic benefits by lowering the risks of adverse effects and avoiding the need for supplemental therapy to manage cancer cachexia. Thus, it is important to raise awareness and encourage the implementation of herbal medicine for combating cancer and suggest an integrated approach in tumor management and treatment.

So far, many studies have shown that plant metabolites can regulate tumor metabolism and growth. They have the ability to control the DNA-damaging factors in cancer cells and regulate DNA transcription in tumors. They possess numerous therapeutic benefits such as anti-obesity effects, cardiovascular effects, antidiabetic effects, immune enhancement, natural antioxidant activity, and anti-inflammatory effects. Plant-derived nutraceuticals are advantageous for the treatment of colon cancer with additional benefit of improving overall health (Prakash et al. 2012). These nutritional compounds have provided better treatment and showed fewer adverse effects (Pandey et al. 2011). The incidence and mortality rates for colon cancer have been increasing in most of the countries, particularly the United States, European, and part of Asian countries. This increasing incidence of colon cancer appears due to changing dietary constituents, physical activity patterns, as well as genetic influences (Center et al. 2009). Reactive oxygen species can cause problems in normal cells. Free radicals such as O2– and OH- may increase normal human colonocyte activity and result in the formation of colon polyps. Natural antioxidants derived from fruits and plant resources can limit the oxidative damage in colon cells and are effective for inducing apoptosis in colon cancer cells. Quercetin belongs to a family of plant-derived flavonoid phytochemicals and is effective for inducing apoptosis in colon cancer cells. Likewise, dietary uses of onion might be able to suppress the proliferation of normal cells. Onion contains high levels of quercetin, which inhibits the effects of colon cancer proliferation in both in vitro and in vivo studies (Potter 1999). Lentinan naturally occurs in the edible mushroom *Lentinus edodes*. The lentinan compound is known as β-1,3-glucan. It is one of the important drugs used as anticancer agents and is used clinically for colon cancer treatment. Lentinan significantly reduces the formation of colon tumors in an animal model. Selenium is an important dietary mineral found in broccoli extract, red wine, dietary fiber, pepper, soya, cloves, fenugreek, ginger, apple, and other vegetables. Selenium is associated with up to a 50% decrease in the risk for colon cancer (Finley et al. 2000). Yellow mustard oil is synthesized by the brassica family of plants and has been examined for its potential anticancer properties. Mustard contains a complex mixture of long-chain polysaccharides that may play a protective role in colon cancer formation (Nobili et al. 2009). Essential oils such as eicosapentaenoic acid (EPA), docosahexaenoic acid (DHA), and omega-3 fatty acids are also used to treat and prevent cancer and cardiac diseases. Particularly, the consumption of fish and fish products reduces the risk of colon cancer progression (Pandey et al. 2010).

16.3 Role of Indian Diet in Colon Cancer Prevention

16.3.1 Role of Spices in Colon Cancer Prevention

In India, spices have been widely used as condiments for thousands of years because of their flavor, taste, and color. Numerous studies have documented the antioxidant, anti-inflammatory, and immunomodulatory effects of spices, which might be related to prevention and treatment of colorectal cancer (Table 16.4). Several spices are potential sources and are used in the prevention and treatment of cancer, such as *Curcuma longa* (turmeric), *Nigella sativa* (black cumin), *Zingiber officinale* (ginger), *Allium sativum* (garlic), *Crocus sativus* (saffron), *Piper nigrum* (black pepper), and *Capsicum annuum* (chili pepper), which contained several important bioactive compounds, such as curcumin, thymoquinone, piperine, and capsaicin and have been used as medicinal plants in folk medicine for the treatment of various diseases. For example, some antioxidants from spices, such as curcumin (turmeric), eugenol (clove), and capsaicin (red pepper), were experimentally evidenced to control cellular oxidative stress due to their antioxidant properties and their capacity to block the production of reactive oxygen species and interfering with signal transduction pathways (Srinivasan 2014; Rubio et al. 2013). Besides, inflammatory processes were modulated by spice compounds such as curcumin and thymoquinone (Ghosh et al. 2014). In addition, spices were sometimes used as a source of alternative antimicrobial strategies, including some spices belonging to the genus *Cinnamomum* (Nabavi et al. 2015). Moreover, the immunomodulatory effects of some spice compounds were confirmed, such as thymoquinone. The antioxidant, anti-inflammatory, and immunomodulatory effects of spices have been confirmed in many studies. Therefore, spices could be used to prevent and treat cancers, because oxidative stress (Chang et al. 2016; Fu et al. 2011; Deng et al. 2012; Bensimon et al. 2016; Li et al. 2013, b, c; Guo et al. 2012), inflammatory stress (Maisonneuve et al. 2016), and immune response (Hegde et al. 2016; Ubillos et al. 2016) have been associated with the genesis, growth, and metastasis of cancers (Li et al. 2013, b, c; Zhou et al. 2016). In fact, epidemiological and experimental evidences have shown that certain spices might lower risks of some cancers (Park et al. 2015; Fridlender et al. 2015; Butt et al. 2013; Zick et al. 2015).

16.3.1.1 Turmeric

Turmeric (*Curcuma longa*) is one of the most commonly used spices in the Indian kitchen; apart from adding that authentic Indian flavor and the vivacious color to our food, it also carries polyphenol curcumin that has been known to reduce and prevent the growth of cancer cells in our body. Curcumin, a polyphenolic compound, is a secondary metabolite isolated from the rhizomes of turmeric and exhibits a number of therapeutic effects, including anticancer properties, via modulating different molecular regulators (Staege et al. 2013; Devassy et al. 2015; Shanmugam et al. 2015).

Table 16.4 Role of spices in CRC prevention

Spices	Constituents	Anticancer effects	References
Turmeric	Curcumin	Preventing aberrant crypt foci, inducing apoptosis, inhibiting cell growth	Carroll et al. (2011); Shehzad et al. (2014); Manikandan et al. (2012); Wang et al. (2015); Guo et al. (2015); Toden et al. (2015); He et al. (2011); Zhou et al. (2016)
Black cumin	Thymoquinone	Attenuating tumor development and growth, inducing apoptosis, inducing autophagic cell death	Jrah-Harzallah et al. (2013); Lang et al. (2013); Kundu et al. (2014a, b); Chen et al. (2015); Gali-Muhtasib et al. (2008); Zhou et al. (2016)
Ginger	Ginger root/leaf extract, 6-gingerol, shogaols	Reducing cell viability and proliferation, inducing apoptosis	Zick et al. (2015); Park et al. (2014a, b); Radhakrishnan et al. (2014); Fu et al. (2014); Citronberg et al. (2013); Zhou et al. (2016)
Garlic	Se-methyl-L-selenocysteine, garlic extract	Inducing apoptosis, suppressing cell proliferation	Tung et al. (2015); Jikihara et al. (2015); Zhou et al. (2016)
Onion	Se-methyl-L-selenocysteine	Inducing apoptosis	Tung et al. (2015); Zhou et al. (2016)
Scallion	Scallion extract	Inhibiting tumor growth	Arulselvan et al. (2012); Zhou et al. (2016)
Saffron	Crocin	Inducing apoptosis	Amin et al. (2015); Zhou et al. (2016)
Black pepper	Piperine	Impairing cell cycle progression and inducing apoptosis	Yaffe et al. (2013); Yaffe et al. (2015); Zhou et al. (2016)
Red chili pepper	Capsaicin	Inhibiting cell proliferation and inducing apoptosis	Clark et al. (2015); Zhou et al. (2016)
Rosemary	Rosemary extract, carnosic acid, diterpenes	Sensitizing cancer cells to 5-FU, inhibiting cell migration, inducing apoptosis	Gonzalez-Vallinas et al. (2013, 2014); Park et al. (2014a, b); Zhou et al. (2016)
Clove	Clove extract	Inhibiting tumor growth and promoting cell cycle arrest and apoptosis	Liu et al. (2014); Zhou et al. (2016)
Galangal	Galangin	Inducing cell death	Ha et al. (2013); Zhou et al. (2016)
Cinnamon	Cinnamaldehyde	Regulating drug-metabolizing genes	Yu et al. (2014); Zhou et al. (2016)
Oregano	Carvacrol	Inhibiting proliferation and inducing apoptosis	Fan et al. (2015); Zhou et al. (2016)

Several clinical studies researched the effects of curcumin on colorectal cancer. Curcumin prevented aberrant crypt foci (ACF) and adenomas in murine models of colorectal carcinogenesis, which were involved in inhibiting the production of mucosal concentrations of pro-carcinogenic eicosanoids 5-hydroxyeicosatetraenoic acid (5-HETE) and prostaglandin E-2 (PGE-2) (Carroll et al. 2011), and curcumin-induced apoptosis could be reversed by PGE-2 in colon cancer cells (Shehzad et al. 2014). In addition, curcumin inhibited the growth of human colon adenocarcinoma cell lines and induced apoptosis (Manikandan et al. 2012) and also led to mitochondrial-mediated apoptosis (Wang et al. 2015).

16.3.1.2 Black Cumin

Nigella sativa, commonly referred to as black cumin, is an annual herb growing in countries bordering the Mediterranean Sea and India and is used as a natural medicine for the treatment of many acute and chronic conditions ranging from fever to intestinal disturbances to cancer (Kundu et al. 2014a, b; Rahmani et al. 2014; Schneider-Stock et al. 2014). Thymoquinone is the predominant bioactive constituent isolated from black seeds of *Nigella sativa* and has been shown to possess antineoplastic activity against multifarious tumors (Attoub et al. 2013).

In colorectal cancer, thymoquinone blocked STAT3 signaling via inhibition of Janus kinase (JAK) 2- and Src-mediated phosphorylation of EGF-R tyrosine kinase, thus inducing apoptosis in human colon cancer cells (Kundu et al. 2014a, b). Besides, thymoquinone led to caspase-independent, autophagic cell death via mitochondrial outer membrane permeability and activation of c-Jun N-terminal kinase (JNK) and p38 in irinotecan-resistant LoVo colon cancer cells (Chen et al. 2015). In addition, in a xenograft model of HCT116 colon cancer cells, thymoquinone significantly inhibited the growth of the tumor cells (Gali-Muhtasib et al. 2008). Thymoquinone (either with pretreatment or posttreatment) could reverse 1,2-dimethylhydrazine (DMH)-induced oxidative stress at initiation and established histological changes and tumor development (Jrah-Harzallah et al. 2013). In another study, tumor growth in ApcMin (Min, multiple intestinal neoplasia) mice was interfered by thymoquinone through inducing tumor cell-specific apoptosis and modulating Wnt signaling via activation of glycogen synthase kinase (GSK)-3β, indicating *Nigella sativa* oil (or thymoquinone) might be useful as nutritional supplement in familial adenomatous polyposis (Lang et al. 2013).

16.3.1.3 Ginger

Ginger (*Zingiber officinale*), a common spice in foods and beverages, is rich in several bioactive phenolics, including nonvolatile pungent compounds such as gingerols, paradols, and shogaols (Karna et al. 2012), which possess antioxidant, anti-inflammatory, antifungal, anti-mycobacterial, and anticarcinogenic proprieties (Haniadka et al. 2012; Pereira et al. 2011). Also, ginger leaf has long been used as a vegetable, tea, and herbal medicine (Park et al. 2014a, b).

Several clinical studies researched the effects of ginger on colorectal cancer. In a study, in people at increased risk of colorectal cancer, proliferation in the normal-appearing colorectal epithelium was reduced, and apoptosis and differentiation were increased by ginger (Citronberg et al. 2013). Elevated tissue levels of PGE-2, whose production is regulated by COX-1 and NAD + -dependent 15-hydroxyprostaglandin dehydrogenase (15-PGDH), are an early event in colorectal cancer. After ginger consumption, colonic COX-1 protein expression in participants at increased risk for colorectal cancer was significantly reduced. In another study focusing on PGE-2, there was a significant decrease in arachidonic acid after ginger treatment in the subjects at normal risk for colorectal cancer. In this way, ginger inhibited COX, and decreased the incidence and multiplicity of adenomas as well as PGE-2 concentrations (Zick et al. 2015).

16.3.1.4 Garlic

Garlic (*Allium sativum*) is a widely used spice, and also a traditional remedy for a variety of ailments. Garlic possesses cancer-preventive potential and significant enhancing effects on the immune system. The potential anticancer effects of garlic are attributed to its metabolic by-products, organosulfur components (Chiavarini et al. 2016; Nicastro et al. 2015). Natural organosulfur compounds exhibit antioxidant and chemo-sensitization properties, and they contain compounds such as diallyl sulfide, diallyl disulfide, diallyl trisulfide, diallyl tetrasulfide, S-allyl mercaptocysteine, and allicin.

Garlic contains natural organoselenium compounds such as selenomethionine and Se-methyl-L-selenocysteine (MseC), which possess lower toxicity and better anticancer activities than inorganic Se. The 80% apoptosis in colo 205 cells was caused by MseC, which was involved in caspase activation, the extrinsic apoptotic pathway, and the regulation of ER stress-induced apoptosis (Tung et al. 2015). Furthermore, aged garlic extract (AGE) produced from fresh garlic for more than 10 months caused a significant decrease of the number of ACF (aberrant crypt foci). The proliferative activities in adenoma and adenocarcinoma lesions were suppressed, without effects on normal colon mucosa. Cell cycle progression and cyclin B1 and cdk1 expression were downregulated via inactivation of NF-κB in the human colorectal cancer cells (Jikihara et al. 2015).

16.3.1.5 Onion and Scallion

Onion (*Allium cepa*) and scallion (*Allium fistulosum*) were also included in *Allium* genus, which showed cancer-preventive effects, attributed to sulfur-containing compounds (Nicastro et al. 2015). Various flavonoids extracted from onions have been shown to offer effective anticancer therapies against colorectal adenocarcinoma cells (Murayyam et al. 2017). One of the constituents in onion, such as quercetin and fisetin, possesses anticancer effects. For instance, quercetin-induced inhibition

of migration and invasion of cancer cells was attributed to downregulating PKC and RhoA by blocking MAPK and PI3K/AKT signaling pathways and NF-κB and uPA, thus suppressing MMP-2 and MMP-9 signaling (Lai et al. 2013). Besides, fisetin showed cancer-preventive effects via modulating the PI3K/Akt/mTOR pathway in cancer cell models (Syed et al. 2013; Khan et al. 2013). Similarly, selenomethionine and Se-methyl-L-selenocysteine were also found in onion, and the effect was like in garlic (Tung et al. 2015). In addition, red onion might decrease the risk of other cancers (Inoue-Choi et al. 2013; Perlman et al. 2013). In another study, scallion extracts showed a significant suppression of colon tumor growth in mice, through inhibiting the key inflammatory markers COX-2 and iNOS and suppressing the expression of various cellular markers involved in tumor apoptosis, proliferation, angiogenesis, and invasion (Arulselvan et al. 2012).

16.3.1.6 Saffron

Saffron (*Crocus sativus*), the dried, dark red flower, is harvested from stigmas of the plant. It is one of the most expensive spices and used as a spice for flavoring and coloring food and as an herbal plant in folk medicine. A number of studies showed that saffron possesses anticancer effects attributed to the bioactive compounds it contained, such as crocin and crocetin. The compounds were abundant in saffron, and they induced apoptosis and inhibited cell proliferation (Zhang et al. 2013; Gutheil et al. 2012).

In both human adenocarcinoma gastric cancer cells and rat model of gastric cancer, crocetin induced apoptosis, suppressed Bcl-2, and upregulated Bax expression in gastric adenocarcinoma cells. Crocetin also reversed 1-methyl-3-nitro-1--nitrosoguanidine-induced changes in serum antioxidant activity and lactate dehydrogenase (Bathaie et al. 2013). Moreover, crocin induced apoptosis in the gastric adenocarcinoma cells. The Bax/Bcl-2 ratio was increased, which indicated that apoptosis was stimulated by crocin and crocin possessed an anticancer effect (Hoshyar et al. 2013). Additionally, crocin induced an autophagy-independent classical programmed cell death in colon cancer cells (Amin et al. 2015).

16.3.1.7 Black Pepper

Black pepper (*Piper nigrum*) is a widely consumed spice, which is also an herb commonly used in folk medicine. Piperine is a major alkaloid constituent of black pepper and exerts antitumor activities in a variety of cancers.

Piperine inhibited the metabolic activity of HRT-18 human rectal adenocarcinoma cells indicating a cytostatic effect. Piperine inhibited cell cycle progression and induced apoptosis (Yaffe et al. 2013). Furthermore, piperine inhibited HT-29 colon carcinoma cell proliferation by causing G1 phase cell cycle arrest. Piperine induced loss of mitochondrial membrane integrity and cleavage of poly (ADP-ribose) polymerase-1. The colony formation and the growth of HT-29 spheroids were inhibited (Yaffe et al. 2015).

16.3.1.8 Red Chili Pepper and Capsaicin

Red chili pepper (*Capsicum annuum*) is a widely consumed spice throughout the world. Capsaicin, the most abundant pungent ingredient of red chili peppers, exerts a potent anticancer effect in various human malignancies including colorectal cancer (Clark et al. 2015).

16.3.1.9 Rosemary

Although widely used in Western diets, especially in the Mediterranean diet, rosemary is also cultivated in India but limited to the cooler parts of the Indian subcontinent. It shows preventative effects against cancer as it contains carnosic acid, carnosol, and rosmanol. Carnosic acid-rich rosemary extract showed anticancer properties in colon cancer, and GCNT3 expression was involved in its antitumor mechanism (Gonzalez-Vallinas, et al. 2014). Moreover, carnosol significantly reduced cell viability and induced apoptosis in human colon cancer via generating ROS, inducing p53, activating caspases, and inhibiting the STAT3 signaling pathway (Park et al. 2014a, b).

16.3.1.10 Clove

Clove (*Syzygium aromaticum*), the sun-dried unopened flower bud from the plant, has been used as a common spice used in India. Eugenol is a major component in clove and several other spices such as basil, cinnamon, and bay leaves. Oleanolic acid is also one of the ingredients of clove extract attributed to its antitumor activity. Moreover, clove may represent a novel therapeutic herb for the treatment of colorectal cancer (Liu et al. 2014).

16.3.1.11 Galangal

Galangal (*Alpinia officinarum*) is a traditional oriental spice, and used in folk medicine. Galangin, a flavonol derived from galangal, exerted anticancer effects on several cancers, including melanoma, hepatoma, and colon cancer cells. For instance, apoptotic pathways in cancer cells might be activated by prolonged endoplasmic reticulum stress. Studies have reported that galangin present in galangal could exert anticancer effects in colon cancer cells (Ha et al. 2013).

16.3.1.12 Coriander

Coriander (*Coriandrum sativum*) is used as a common culinary spice and medicinal herb used in Indian cuisine. Linalool, abundant in coriander, is one of the active components responsible for its anticancer effect. The anticancer effect of linalool was through inducing oxidative stress (Jana et al. 2014).

16.3.1.13 Cinnamon

Cinnamon (*Cinnamomum cassia*), a traditional oriental medicinal herb, is also widely used as a spice in India. Cinnamaldehyde, the bioactive component isolated from the stem bark of cinnamon, exerted an anticancer activity against various cancers. Cinnamon polyphenols in combination with chemotherapeutic agents (5-FU, OXA) exerted a synergistic effect on cytotoxicity in colorectal carcinoma cells by suppressing the expression of BRCA1, TOPO1, ERCC1, and TS mRNA (Kim et al. 2015).

16.3.1.14 Oregano

The ancient herbal records say that oregano (*Origanum vulgare*) was referred to as wild marjoram (https://www.indianmirror.com/ayurveda/oregano.html), is a widely used spice, and contains carvacrol, thymol, and many other anticancer components. For instance, β-caryophyllene oxide, a sesquiterpene isolated from the essential oils of medicinal plants including oregano, showed an anticancer effect through blocking the STAT3 activation pathway in cancer cells (Kim et al. 2014). Moreover, carvacrol induced apoptosis via the mitochondrial apoptotic pathway and the MAPK and PI3K/Akt signaling pathways in colon cancer cells (Fan et al. 2015).

16.3.1.15 Cardamom

Cardamom (*Elettaria cardamom*), a dietary phytoproduct, is used as a spice and exerts anticancerous properties. A group of Indian scientists have found that cardamonin, a chemical found in cardamom and other edible plants such as ginger and peppercorn, is effective in preventing and controlling colorectal cancer in mice (https://www.thehindubusinessline.com). Cardamonin modulates certain microRNAs that collectively regulate the reactive oxygen production and thus help in controlling colorectal cancer.

16.3.2 Role of Condiments in Colon Cancer Prevention

Indian condiments like asafoetida, bay leaf, black peppercorn, black salt, cardamom powder, carom seeds, coriander powder, coriander seeds, cumin seeds, curry leaves, dry fenugreek leaves, fennel seeds, ginger powder, Himalayan salt, mace, mustard seeds, nigella seeds, nutmeg, poppy seeds, red chili powder, sesame seeds, star anise, sunflower seeds, turmeric, etc. and various Indian pickles and sauces like Branston pickled chutney, chick pea chutney, coconut chutney, coriander chutney, curd chutney, dried ginger chutney, garlic chutney, gooseberry chutney, green mango chutney, hog plum chutney, mango ginger chutney, mint chutney, papaya chutney, peanut chutney, pineapple chutney, ridge gourd chutney, stink bean chutney, tamarind chutney, tomato chutney, wild melon chutney, etc., along with

cooking oils like coconut oil, corn oil, cottonseed oil, flaxseed oil/linseed oil, mustard oil, niger seed oil, olive oil, palm oil, peanut oil, rapeseed oil, rice bran oil, safflower oil, sesame oil, soybean oil, sunflower oil, etc. have various health benefits with antitumor and antimetastatic effects on various cancers.

16.3.2.1 Honey

Honey was the first sweet food tasted by the ancient Indian inhabiting rock shelters and forests. Eugenol is a natural compound which is derived from honey and is present in some plant extracts including clove oil, cinnamon, Flos Magnolia, citrus, and balm. Eugenol exhibits novel medical applications for curing various chronic diseases. It promotes apoptosis in colon cancer cells (Seeram et al. 2006). Eugenol is a potential natural drug against colorectal cancer. Eugenol stimulates cell division in sub-G1 phase inducing apoptosis in a regular time-dependent manner. It acts as a transducer of an apoptosis signal to control the production of nonprotein thiols and matrix metalloproteinase (MMP). Eugenol-treated colon cancer cells demonstrated increased p53 activation and proline-rich acidic protein (PRAP) cleavage (Jaganathan et al. 2011).

16.3.2.2 Nuts and Seeds

A diet rich in nuts is also associated with reduced rate of cancer recurrence and death in patients with stage 3 cancer. Nuts and seeds are rich in omega-3 fatty acids. Fatty acids are long-chain hydrocarbons which may vary from 10 to 30 carbons and are a component of lipids. Fatty acids such as saturated fatty acids and unsaturated fatty acids are found in flaxseed, chia seeds, and walnuts. Omega-3 PUFAs have been broadly studied in clinical and pathological conditions. The consumption of omega-3 PUFAs has been correlated with human health benefits. Omega-3 fatty acids have many clinical benefits, including reducing the risk of tumor growth and metastasis. The highest level of omega-3 fatty acids can alter eicosanoid synthesis and have anti-catabolic effects. The supplementation of these essential fatty acids, eicosapentaenoic acids (EPA) and docosahexaenoic acid (DHA), protects against colorectal cancer (Larsson et al. 2004). Hence, it controls weight loss in cancer patients, regulates cytokine production, and stabilizes the energy metabolism. Several clinical studies evaluated high-fat and low-carbohydrate fish oil supplement as a potential therapeutic for colon cancer. Omega-3 fatty acid in fish oil supplements stimulates the immune response and enhances apoptosis in cancer cells (Cockbain et al. 2012). Suggested that n-3 fatty acids have antitumor effects during the initiation stages of colon carcinoma. The omega-3 fatty acids reduce the proliferation of early-stage colonic cancers, which may reduce the progression of colorectal polyps and may help protect high-risk individuals from colon cancer.

16.3.2.3 Tea and Coffee

Higher levels of coffee consumption are associated with lower recurrence rate. One study showed that a cup of coffee each day reduces mortality rate by as much as 20% for patients with stage 3 colorectal cancer (https://www.mountelizabeth.com). Consumption of herbal tea was also associated with a significantly decreased risk of distal colon cancer.

16.3.2.4 Salads

Green leafy vegetables are rich in vitamins and play an essential role in cancer prevention and treatment. Folic acid plays a major role in DNA methylation and DNA synthesis. It conjugates with vitamins B6 and B12 in the single-carbon methyl cycle. Vitamin B complex treatment was initiated to reduce the risk of colon, rectal, and breast cancer (Lee et al. 2003). Similarly, vitamin D receptor molecules are highly expressed in colon cancer cells and may control the abnormal metastasis and regulate the cell death mechanism in colon cells (Tangpricha et al. 2001).

16.4 Role of Other Commonly Used Indian Foods in Colon Cancer Prevention

16.4.1 Tamarind

Tamarind (*Tamarindus indica*) bean pulp has been used as a condiment in Indian culinary cooking since time immemorial, and its tangy taste is well accepted by most Indians. Various healing properties of tamarind seeds such as digestive, laxative, expectorant, hypoglycemic, antioxidant, antiatherosclerotic, antimutagenic, anti-inflammatory, hypolipidemic, antidiabetic, and carminative have been reported (Hemshekhar et al. 2011). The seeds are known to be a rich source of many polyphenolic bioactive compounds, triterpenes, and polysaccharides, which have healing activities for various human diseases. The use of tamarind seeds has also been suggested by Martinello et al. (2017) to have a chemopreventive activity against the development of colon carcinogenesis.

16.4.2 Pomegranate

Pomegranate (*Punica granatum)* has been valued as the best source of phenolic compounds in the human diet since ancient times, mainly because of the biological effects it exerts through the free radical scavenging capabilities (Sidhu and Zafar 2020). Pomegranate is one such fruit rich in many bioactive compounds that offer health benefits such as anticarcinogenic and immune-boosting properties.

Pomegranate fruit shows antioxidant, anti-inflammatory, antiangiogenetic, antiproliferative, antimetastatic, antiinvasive, and apoptotic properties. Thus, the consumption of this fruit would assist in leading a healthy life protected from cancers (Khwairakpam et al. 2018). Pomegranate showed suppression of colon cancer through downregulation of Wnt/β-catenin in a rat model (Ahmed et al. 2017; Nunez-Sanchez et al. 2017).

16.4.3 Amla

Amla (*Phyllanthus emblica* L.) has been used in India for many centuries, and the usefulness of its fruits, leaves, bark, and roots has been well documented for its medicinal value (Pammei et al. 2019). The fruit is not only the richest source of ascorbic acid but also an excellent source of pectin, many minerals, and phenolic antioxidants (Alkndari et al. 2019a, b). Amla fruit possesses many health-promoting properties such as anticancer, antioxidant, anti-inflammatory, antimicrobial, immunomodulator, and cytoprotective properties.

16.4.4 Sugar Beet

Beet (*Beta vulgaris*) is known to be a rich source of several bioactive compounds such as betaine, betacyanins, betanins, vulgaxanthins, betaxanthins, polyphenols, flavonoids, many vitamins (thiamine, riboflavin, folic acid, biotin, pyridoxine, ascorbic acid), pectin, soluble fiber, and many minerals. All these bioactives are known to offer protection against cancer as well as delay the metastasis (Blazovics and Sardi 2018). The anthocyanins present in beetroot also reduced the oxidative stress triggered by hydrogen peroxide in CaCo-2 cells. The anti-inflammatory action by betalains was shown by decreasing cyclooxygenase-2 and interleukin-8 mRNA expression after lipopolysaccharide induction in CaCo-2 cells, thus showing its potential as a chemopreventive tool against colon cancer.

16.4.5 Bitter Gourd

Bitter gourd or bitter melon (*Momordica charantia*) is a widely distributed vegetable in Asia, Africa, and some South American countries. Though bitter, it is a very common and popular vegetable in Asian countries simply because of its rich taste and nutritional value. This vegetable has been valued for its antidiabetic, anti-HIV, antioxidant, anticancer, antibacterial, immunomodulatory, anti-obesity, and anti-inflammatory properties. Bitter gourd seed oil has been reported to be rich (a 60% level) in conjugated linolenic acid (CLA), which induces apoptosis and was found to upregulate the GADD45, p53, and PPARγ in human colon cancer CaCo-2 cells in a dose-dependent manner (Yasui et al. 2005).

16.4.6 Moringa

Moringa (*Moringa oleifera)*, a native of the Indian subcontinent, is an edible evergreen plant that can grow in a wide range of climatic and soil conditions. Out of 13 cultivars, *M. oleifera* is the most important plant for its phytochemicals and pharmacological properties related to human health (Ma et al. 2018). Apart from other phytochemicals, the Moringa plant has been valued for its glucosinolates and their derivative compounds that have been found to be active against many diseases, including cancer (Ribaudo et al. 2019). A novel polysaccharide (MOP-2) consisting of arabinose (35.8%), glucose (6.67%), and galactose (57.53%), having immunostimulatory and MOP-2, enhanced the proliferation of macrophages and promoted the secretion of ROS, nitric oxide, interleukin-6, as well as tumor necrosis factor-α through the activation of mRNA expressions of iNOS, IL-6, and TNF-α (Dong et al. 2018). Different parts such as roots, leaves, bark, and drumsticks have enormous properties in nutrition, medicine, or industrial utilization (Liu et al. 2018a, b). A novel arabinogalactan (MOP-1) has been isolated from the leaves of *M. oleifera* by He et al. (2018), who also elucidated its structure (it has arabinose, rhamnose, and galactose in molar ratios of 1:7.32:12.12) and found that the leaves possess tremendous antioxidant activity. Elwan et al. (2018) investigated the ethanolic extracts of Moringa leaves as a protective and therapeutic agent against the damage induced by high acute doses of ionizing radiation. The *M. oleifera* seeds are known to contain lectins, which are carbohydrate-binding proteins with beneficial biological properties including cytotoxicity to B16-F10 melanoma cancer cells (Luz et al. 2017). In a later study, Shu et al. (2018) reported that *M. oleifera* seed extract residue suppressed the metastasis of cancer cells.

16.5 Dietary Phytochemicals in Colorectal Cancer Prevention

Phytochemicals are non-nutritive secondary metabolites derived from plants, often with health-promoting and disease-preventive properties, mainly found in fruits, vegetables, grains, herbs, spices, and other plant foods. On the basis of epidemiological as well as preclinical and clinical research evidence, the consumption of fruits and vegetables exerts health-promoting effects against different types of tumors (Gonzalez-Vallinas et al. 2013). About 70–90% of CRC are correlated with dietary factors, and diet optimization can avoid most of the cases. Dietary phytochemicals have been implicated in an extensive range of anticancer activities such as anti-proliferation, cell cycle blockage, DNA repair alteration, apoptosis induction, anti-inflammation, activation of tumor-suppressor genes and suppression of oncogenes, regulation of the levels of hormonal and growth factors, and inhibition of invasion, angiogenesis, and metastasis (Table 16.5). It has been found that phytochemicals can modulate key cellular signaling pathways by targeting different stages of CRC (initiation to progression), and research endeavors have centered on the roles of phytochemicals in signaling cascades that are presumed to induce

Table 16.5 Functions and molecular targets of dietary phytochemicals against CRC

Phytochemicals			Indian dietary source	Function and molecular targets	References
Polyphenols	Flavonoids	Quercetin	Indian gooseberry, neem, radish, onion, garlic, dill weed, coriander, capsicum, shatavari (Indian asparagus), okra, moringa, turmeric, berries, peppers, citrus fruits, apples, grapes, green leafy vegetables, oregano, honey, tea, coffee, etc.	Induces apoptosis by ↓MMP, ↑ROS, ↑SIRT-2/p-AMPK/↓p-mTOR, ↑Bax, p53, caspase-3, ↑c-PARP, caspase-3, caspase-9, ↓Bcl-2, Bcl-xL, NF-κB Induces cell proliferation Arrests cell cycle at G0/G1 phase Suppresses metastasis by ↑p-Erk, p-JNK, p-p38 MAPK Decreases tumor nodules	Kim et al. 2014); Afrin et al. (2020); https://www.quercetin.com/natural-medicine/traditional-medicine-herbs-with-quercetin; https://www.superfoodly.com/quercetin-foods
		Anthocyanin	Beetroot, corn, grapes, cauliflower, cranberry, blueberry, etc.	Suppresses migration and invasion by ↓claudin, ↑p38MAPK, ↓PI3K/Akt, ↓MMP-2, MMP-9 Demethylates tumor-suppressor genes by ↓DNMT1, DNMT3B, β-catenin, c-MYC, Wnt Induces apoptosis by ↑c-PARP, caspase-3, Bax/Bcl-2 Arrests cell cycle at G0/G1phase by ↓cyclin E, cyclin D, ↑p21, p27 Inhibits cell proliferation by ↓Wnt/β-catenin, c-MYC, cyclin D1, ↑ROS Induces apoptosis by ↓survivin, cIAP-2, and XIAP Decreases inflammation by ↓TNF-α, IL-1β, IL-6, and NF-κB	Afrin et al. (2020); https://www.ncbi.nlm.nih.gov
		Genistein	Green lentil, soya bean, chickpeas, legumes, sunflower, barley meal, broccoli, cauliflower, etc.	Inhibits cell proliferation by ↓EGF-R, ↓p-p38 MAPK, ↓cyclin B1, ChK2 Suppresses cancer progression by ↓PCNA, ↑Nrf2, HO-1, ↓β-catenin, stem cell marker Suppresses pre-neoplasis by ↓Wnt/β-catenin, cyclin D1, c-Myc Induces apoptosis by ↓cdc2, cdc25A, ↑ATM/p53, p21waf1/cip1, GADD45α Suppresses migration and invasion by ↓MMP-2 Suppresses metastasis by ↓MMP-2, FLT4, CD34 Arrests cell cycle at G2/M phase Suppresses cell proliferation by ↓DNA topoisomerase II activity Induces epigenetic modification by ↑DKK1, ↓HDAC1	Afrin et al. (2020); https://www.zerobreastcancer.org

		EGCG (epigallocatechin-3-gallate)	Nuts, cranberry, blueberry, apple, tea, etc.	Inhibits cell proliferation by ↓PI3K/Akt, ↓Wnt, cyclin D1, c-MYC, ↓Erk1/2, NF-κB, Induces apoptosis by ↑c-PARP, caspase-9, ↑p-Erk1/2, p-JNK1/2, p-p38MAPK, ↑Bax, ↓Bcl-2, ↑caspase-9, PARP, ROS Suppresses migration and invasion by ↑AMPK, ↓MMP-2, VEGF Arrests cell cycle at G0/G1 phase Induces epigenetic modification by ↓RXRα, β-catenin, cyclin D1 Controls DNA methylation by ↓DNMT3A, HDAC3	Afrin et al. (2020); https://www.healthline.com
		Silibinin	Sunflower, turmeric, etc.	Induces apoptosis by ↑DNA fragmentation, ↑caspase-3, caspase-8, caspase-9, caspase -10, Bid, cyto-c, TRAIL, DR4/DR5, ↓Mcl-1, XIAP, ↑NAG-1, EGR-1 Inhibits cell proliferation -↓IL-4, IL-6, p-STAT3, NF-κB Suppresses migration and invasion -↓MMP-2, JNK, AP-1 Decreases tumor growth by ↓ACF proliferation and formation Induces apoptosis by ↓Bcl-2, ↑Bax Suppresses inflammation by ↓MMP-7, TNF-α, IL1β Decreases adenoma formation by ↑CDX2 Induces epigenetic modification by ↓DNMT	Afrin et al. (2020); https://healthyliving.azcentral.com/what-are-the-dangers-of-eating-outdated-peanut-butter-12528044.html
		Kaempferol	Turnip, onion, cucumber, etc.	Inhibits cell proliferation by ↓IGF-1R, ErbB3, p-PI3K/Akt, p-Erk/12 Induces apoptosis by ↑c-caspase-3, c-caspase-7, c-caspase-9, PARP, Bik, Bad, ↓Bcl-xL, ↑FasL, cyto-c Arrests cell cycle at G1 and G2/M phase by ↓CDK2, CDK4, Cdc25C, Cdc2, cyclin B1, cyclins D1, cyclin E, cyclin A, p-Rb Prevents oxidative damage -↓Lipid peroxidation, ↑CAT, SOD, GPx Induces epigenetic modification by ↑hyperacetylation of histone complex H3	Afrin et al. (2020); http://www.nutrition.merschat.com/foods-by-nutrient.cgi?Nutr_No=786&Measure=cm1&page=1
	Phenolic acid and derivatives	Caffeic acid	Coffee, rice, cumin, ginger, nutmeg, brinjal, etc.	Induces apoptosis by ↑ROS, ↓MMP, ↑c-PARP Arrests cell cycle at sub-G1 phase Controls DNA damage by ↓DNA topoisomerase II activity	Afrin et al. (2020); http://phenol-explorer.eu/contents/polyphenol/457

(continued)

Table 16.5 (continued)

Phytochemicals			Indian dietary source	Function and molecular targets	References
		Rosmarinic acid	Basil	Induces apoptosis by ↑Fas, FasL, caspase-3, caspase-8, caspase-9, Bid, Bax, ↑Cyt-c, AIF, c-PARP, DFF-45 Improves premalignant lesion and antioxidant status by ↓ACF formation and ↑antioxidant status Inhibits cell proliferation by ↓PCNA Suppresses inflammation by ↓COX-2, TNF-α, IL-6, NF-κB Reduces tumor incidence and multiplicity by ↓polyp Induces apoptosis by ↑antioxidant status, ↓phase I and ↑phase II enzyme, ↑p53, caspase-3, caspase-9, Bax, ↓Bcl-2	Afrin et al. (2020); http://phenol-explorer.eu/contents/polyphenol/461
		Ellagic acid	Pomegranate	Inhibits cell proliferation by ↓PCNA, K-ras, p-PI3K/Akt, ↓PCNA, cyclin D1, p-PI3K/Akt Induces apoptosis by ↑P53, ↑caspase-8, ↑ROS, Bax, Cyto-c, ↓Bcl-2, ↑caspase-3, ↑DNA fragmentation Arrests cell cycle at G1 phase Anticancer effects by ↑antioxidant status, ↓ACF formation, c-MYC Suppresses angiogenesis by ↓MMP-2 and MMP-9 Decreases detoxification by ↓phase I and ↑phase II enzyme Decreases inflammation by COX-2, iNOS, ↓p-p38MAPK, NF-κB, and p-STAT3	Afrin et al. (2020); https://www.healwithfood.org/foods-that-contain/pelagic-acid-high-amounts.php
		Gallic acid	Cloves, brinjal, banana, etc.	Inhibits cell proliferation by ↓NF-kB, AP-1, STAT-1, OCT-1, ↓CSC markers, ↓Notch1, Wnt/β-catenin, ↓PCNA, K-ras, p-PI3K/Akt Arrests cell cycle at G0/G1 phase Induces apoptosis by ↑P53, ↓cyclin D1, ↑caspase-3, ↑ROS, ↓MMP, ↑ROS, Bax, Cyto-c, ↓Bcl-2, ↑caspase-3, ↑DNA fragmentation Decreases inflammation by ↓iNOS, COX-2, IL-6, ↓p-STAT3Y705, p-IκB, p65-NF-κB Anticancer effects by ↑antioxidant status, ↓ACF formation, c-MYC Suppresses angiogenesis by ↓MMP-2 and MMP-9 Decreases detoxification by ↓phase I and ↑phase II enzyme Decreases inflammation by COX-2, iNOS	Afrin et al. (2020); http://phenol-explorer.eu/contents/polyphenol/413

	Resveratrol	Jackfruit, Indian mulberry, etc.	Inhibits cell proliferation by ↓NF-κB, ↑Sirt1 Suppresses migration and invasion by ↓MMP-9, CXCR4 Inhibits cell proliferation by ↓Ras, Raf, MEK, Erk1/2 Induces apoptosis by ↑Bak1, Bok, Bik, Noxa, Bad, Bax, p53, Apaf1, ↓Bcl-2, Bcl-xL, Bag1, ↑c-caspase-3, c-caspase-7, c-caspase-9, ↑c-PARP, caspase-7, caspase-9 Inhibits cell proliferation by ↓PI3K/Akt, Wnt/β-catenin Suppresses tumor growth Inhibits cell proliferation by ↓Wnt/β-catenin, T brachyury, conductin, cyclin D1, disrupt TCF/ β-catenin interaction Arrests cell cycle at G1/S phase by ↓cyclin D1, CDK2, CDK4, PCNA, p21 Anti-inflammatory effects by ↓iNOS, TLR-4, p-IκB, NF-κB Suppresses invasion and metastasis by ↓Wnt/β-catenin, c-MYC, MMP-7	Afrin et al. (2020); https://www.webmd.com/diet/qa/how-can-you-get-resveratrol-naturally-from-foods; https://www.superfoodly.com/resveratrol-foods-supplements
	Curcumin	Turmeric	Inhibits cell proliferation by ↓PI3K/Akt, ↓EIF2, eIF4/p70S6K, p-mTOR, ↓mTORC1, ↑p-Erk1/2, p-AMPKα1, ↓p-MEK, ↓Wnt, β-catenin, TCF4, ↑axin Induces apoptosis by ↑caspase-3, cyto-c, Bax, ↓Bcl-2, ↑GRP78, ↑ROS, ↓Prp4, Induces autophagy by ↑TFEB lysosomal pathway, ↑LC3-II, ↓p62, Akt, mTOR Suppresses invasion by ↑AMPK, ↓p65 NF-κB, uPA, MMP-9 Suppresses inflammation by ↓COX-2, iNOS, NO, TNF-α, ↓colonic proliferation, ↓COX-2, TNF-α, IL-6, NF-κB, ↑AMPK Decreases inflammation by ↓cyclinD1, CDK4, p-STAT3 Improves adipocytokine levels by ↓leptin Reduces oxidative and nitrosative stress -Reduces arginase activity by ↓ACF formation Induces epigenetic modification by ↓CpG methylation, ↓Histone deacetylases subtypes, DNA methyltransferases, ↑miR-491, ↓PEG10, Wnt/β-catenin, ↓miR-20a, miR-27a, miR-17-5p	Guo et al. (2015); Afrin et al. (2020); https://www.naturalpedia.com/curcumin-sources-health-benefits-and-uses.html

(continued)

Table 16.5 (continued)

Phytochemicals		Indian dietary source	Function and molecular targets	References
Terpenoids	Ursolic acid	Holy basil	Inhibits cell proliferation by ↓SHH, p-STAT3, p-Akt, p-p70S6K, ↓PCNA, p-STAT3, p-Erk, p-JNK, p-p38MAPK, ↓β-catenin, cyclin D1, c-MYC, axin2, ↓p-PI3K/Akt, p-Erk, p-mTOR, COX-2, PGE-2, NF-κB Suppresses angiogenesis by ↓VEGF-A, bFGF Decreases tumor volume Induces apoptosis by ↑Bax/Bcl-2, ↑caspase-3, c-PARP, Bax, ↓Bcl-2, survivin Arrests cell cycle at G1/S phase Reduces tumor growth by ↓cyclin D1, CDK4, ↑p21 Suppresses migration by ↑c-PARP, caspase-3, caspase-9, cyto-c, ↓MMP-9, ↑CDH1	Afrin et al. (2020); https://ursolicacid.com/botanical.html
	Betulinic acid	Himalayan birch	Induces epigenetic modification by ↓Sp1, Sp3, Sp4 Decreases tumor growth by ↓survivin, VEGF, p65-NF-B, EGF-R, cyclin D1, pituitary tumor-transforming gene-1, ↑ROS, miR-27a, ZBTB10 Induces apoptosis by ↑ROS, DNA fragmentation, ↓MMP, ↑caspase-3, caspase-9, Bax, Bad, ↓Bcl-2, Bcl-xl Induces autophagy by ↑Beclin1, Atg 3, Atg 5, Atg 7, Atg 5–12, ↓p62, ↑LC3B, Bax, ↓proteasomal degradation	Afrin et al. (2020); Dutta et al. (2016); https://www.researchgate.net

Organosulfur compounds	Sulforaphene	Mustard	Induces apoptosis by ↑c-PARP, p-MK2, p-p38MAPK, p-JNK, −↑ROS, Ca2+, ↓MMP, ↑cyto-c, DR4, DR5, TRAIL, ↓Bcl-2, ↑Bax, caspase-3, caspase-7, caspase-9 Arrests cell cycle at G2/M phase by ↑calpain1, ATF6α, ATF6β, GADD153, GRP78 Reduces microtubule polymerization by -↑ROS, ↓glutathione Decreases tumor growth Induces apoptosis by ↑Bax, ↓Bcl-2 Inhibits cell proliferation by ↑p27KIPI, ↓SKP2 Induces ER stress by caspase-3, caspase-4, caspase-8, caspase-9, c-PARP Induces autophagy by ↑LC3-II, UGT1A, ↑Nrf2, hPXR Improves drug delivery system by ↓EGF-R degradation Suppresses angiogenesis and migration by ↓HIF-1α, VEGF Induces epigenetic modification by ↓miR-21, HDAC1, ↓hTERT mRNA, telomerase protein, enzymatic levels, controls pseudogene, NMRAL2P, NQ01 induction	Kim et al. (2015); Afrin et al. (2020) https://drjohnday.com/sulforaphane-broccoli-sprouts-best-food-prevent-cancer-heart-disease
	Indole-3-carbinol	Mustard greens, turnip, etc.	Inhibits cell proliferation by ↓Akt, mTOR, GSK3β Induces apoptosis by ↑p53, p21, ↓Bcl-2, ASK1, ↑NDRG1 Decreases tumor growth Suppresses cell growth by ↓cyclin D1, cyclin A, NF-κB Induces epigenetic modification by ↑Aryl hydrocarbon receptor activity, CYP1A1 mRNA expression	Afrin et al. (2020); https://nootriment.com/indole-3-carbinol-foods

chemopreventive activities (Gonzalez-Vallinas et al. 2013). As a result, scientists have paid great attention to establish and build up preventative strategies and treatments to decrease the incidence of CRC.

16.6 Conclusion

The low incidence of colorectal cancer in India compared to the Western world is believed to be due to the predominantly vegetarian diet with high fiber and low meat intake. Ayurveda, the oldest Indian indigenous medicine system of plant-based drugs, seems to be a most promising complementary approach and alternative medicine for the management of cancer as the modern therapy is known to produce drug-induced toxic side effects. Scientific validation is the need of the hour as this will further provide a helping hand in the management of colorectal cancer.

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Chapter 17
Extracellular Vesicles in Colorectal Cancer Progression, Metastasis, Diagnosis, and Therapy

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Abstract A few years ago, extracellular vesicles (EVs) came into light for their role in intercommunication between the tumor cells, transferring oncogenic molecules, and the development of cancer. The EVs are released by almost all types of body cells and can be isolated easily from most of the body fluids. They contain molecular cargos like various types of RNAs, proteins, and growth factors that affect the physiology of the receiving cell. Due to their small size, the ability to easily cross biological barriers, and less immunogenicity and toxicity, they can be bioengineered and used as a vehicle for drugs for targeted delivery to cancer cells. This chapter will elaborate on the association of EVs with colorectal cancer regarding their role in development and metastasis. The importance of EVs as a prognostic and diagnostic marker of CRC will also be detailed. Moreover, their role in cancer therapeutics and drug resistance will be discussed.

Keywords Extracellular vesicle · Cell-cell communication · Drug resistance · Metastasis · Cancer diagnosis · Cancer therapy

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17.1 Extracellular Vesicles

Extracellular vesicles (EVs) are cell-derived vesicles, which can be released from various types of cells in different body fluids. EVs are rich in lipids, polypeptides/proteins, nucleic acids, and other bioactive substances. EVs are involved in various physiological and pathological processes including cell cycle, apoptosis, angiogenesis, thrombosis formation, immunity, inflammation, fibrosis, and tumor development (Andrei-Dennis Voichitoiu et al. 2019). EVs can be classified into the following types (Cufaro et al. 2019).

17.1.1 Exosomes

Exosomes are 40–100 nm in size with a cup-shaped morphology. They are secreted in almost all types of biological fluids, viz., blood, urine, saliva, tears, etc. (Cufaro et al. 2019). Among cancer patients, two types of exosomes are observed: one released by normal cells and another by cancer cells (Andrei-Dennis Voichitoiu et al. 2019). There are two known pathways of biogenesis of exosomes. The first pathway involves the origin of exosomes via the endosomal pathway within multivesicular bodies (MVBs) or multivesicular endosomes (MVEs) and fusion with the plasma membrane. The second pathway includes direct formation from the plasma membrane (Cufaro et al. 2019). Exosome membranes are rich in phospholipids, cholesterol, sphingomyelin, and ceramide. Protein markers of exosomes include Alix, CD63, CD9, CD81, etc. (Cufaro et al. 2019).

17.1.2 Microvesicles (MVs) or Ectosomes

These are large pleomorphic vesicles of 100–1000 nm diameter, which arise by budding/blebbing from the plasma membrane and later detached from the cell surface (Andrei-Dennis Voichitoiu et al. 2019). The shedding takes place at different rates in various types of cells, and even resting cells shed MVs at a slower rate (Kalra et al. 2016). The molecular composition of MVs is yet to be understood, but they are observed to be enriched in certain matrix metalloproteinases (MMPs), glycoproteins (e.g., GPIb), selectins, β-integrins, ARF-6 (ADP-ribosylation factor 6), CD40 (cluster of differentiation 40), lineage markers, β-actin, etc., depending on the types of cells (Andrei-Dennis Voichitoiu et al. 2019; Kalra et al. 2016). MVs also have an abundant exposure of phosphatidylserine on their surface (Théry et al. 2009).

17.1.3 Large Oncosomes (LOs)

There is another category of EVs, which is very similar to MVs in biogenesis, membrane lipid composition, and expression of markers like ARF-6 and MMPs, but the diameter is more than 1000 nm. These entities are called oncosomes. Oncosomes are shed from aggressive cancer cells, but they are rarely observed in the case of benign tumors. Oncosomes are rich in mRNA, miRNA, genomic DNA of tumor cells, enzymes of cancer metabolism, and proteins related to cancer progression, angiogenesis, and cell migration (Andrei-Dennis Voichitoiu et al. 2019; Cufaro et al. 2019). Oncosomes contain specific protein cargos and thus can serve as a biomarker of cancer (Minciacchi et al. 2015).

17.1.4 Apoptotic Bodies (ABs)

These are morphologically heterogeneous vesicles of 50–500 nm diameter and are extruded from the cells undergoing apoptosis. Apoptosis involves systematic disassembling of the cells in a coordinated manner, and the cell remnants are enclosed into apoptotic bodies for disposal. ABs may possess intact organelles, histones, genomic DNA, etc. The biogenesis of ABs includes outward blebbing of the plasma membrane in a caspase-3, myosin light chain, and actin-mediated manner (Kalra et al. 2016). The membrane of ABs contains high levels of phosphatidylserine and histones that can serve as a protein marker of ABs (Cufaro et al. 2019).

17.2 Isolation, Detection, and Analysis of Exosome

EVs can be isolated from various types of cells and characterized in healthy and stressed conditions. The major methods for EV isolation are ultracentrifugation with sucrose gradient and the immune-bead isolation technique like magnetic-activated cell sorting (Li et al. 2017b; Zhang et al. 2015). Commercial kits are also available for the isolation of EVs (Zhang et al. 2015). Electron microscopy, Western blot, and FACS (fluorescence-activated cell sorting) are mostly utilized to characterize the isolated EVs based on their properties, viz., structure, diameter, and biochemical markers (Li et al. 2017b; Zhang et al. 2015). No rigorous methods are available for the determination of the concentration of EVs. The researchers have to depend on evaluation of protein concentration or nanoparticle tracking analysis (Li et al. 2017b; Zhang et al. 2015). qRT-PCR, nucleic acid sequencing, Western blot, and ELISA are used for the recognition of RNA and protein inside EVs (Li et al. 2017b; Zhang et al. 2015). The International Society for Extracellular Vesicles (ISEV) has recently released minimum experimental conditions for the definition of EVs and their behavior (Lötvall et al. 2014).

17.3 Role of EVs in Cancer

EVs have several implications in the advancement of cancer and metastasis. Since EVs are critical in assisting cell-to-cell communication even in normal cells and ease many biological functions, they are pivotal in the intercellular association of tumor cells. EVs contain several biologically active molecules within that can interfere with the fundamental processes and metabolism of the recipient cells. These molecules can target fibroblasts, endothelial cells, immune cells, and extracellular matrix (ECM) (Cufaro et al. 2019). Thus, cellular interactions among cancer cells are one of the key regulators of their proliferation, invasiveness, vascular remodeling, and metastasis. EVs also contain cell-specific receptors on their surface, which deliver them to a particular target cell only. The EVs derived from tumor cells have been demonstrated to contain TGF-β (transforming growth factor β), fibronectin, etc. that can convert normal fibroblasts into cancer-associated fibroblasts (Becker et al. 2016; Minciacchi et al. 2015). The EVs obtained from brain tumor cells have been shown to possess tumorigenic EGFRvIII (epidermal growth factor receptor variant III), which is specific to glioblastoma (Al-Nedawi et al. 2008). Exosomes derived from oncogenic Ras-transformed kidney cells can cause epithelial-mesenchymal transition (EMT) in the recipient cells (Tauro et al. 2013b). Transfer of oncogenic mutant KRAS (V-Ki-ras2 Kirsten rat sarcoma viral oncogene homolog) packed in exosome from colorectal cancer cells to onco-transformed cells can also aggravate their three-dimensional growth (Beckler et al. 2013). Further, MMPs (MMP-2 and MMP-9) have been observed in oncosomes originated from prostate cancer cells that indicates their possible role in tumor cell invasiveness (Li and Nabet 2019). It has also been shown that LOs in blood plasma of prostate cancer patients contain most of the genomic DNA with cancer-specific modifications (Vagner et al. 2018), and plasma-circulating tumor-derived DNA is enough to recognize the alterations in metastatic tissues, indicating its potential to be used as a DNA marker for “metastatic castration-resistant prostate cancer” (Wyatt et al. 2017). MicroRNA and retrotransposon RNA transcripts (viz., LINE-1 and Alu retrotransposon) have also been observed in EVs derived from various types of cancer cells (Balaj et al. 2011; Ohshima et al. 2010).

17.4 Colorectal Cancer (CRC)

According to GLOBOCAN 2018, CRC is the third most diagnosed type of cancer in the world (Bray et al. 2018). It is responsible for over 8% of all deaths every year worldwide (Ma et al. 2018) and is the third most leading causes of mortality (Ydy et al. 2019). It is more prevalent in males as compared to females and is more common in developed countries than the developing ones. It is the second deadliest cancer globally with an estimate of 8,81,000 casualties in 2018. Among these, colon cancer is the fifth deadliest and rectal cancer is the tenth (Bray et al. 2018; Rawla

et al. 2019). Most of the CRC progress through a series of structural changes. Above 95% of CRC is adenocarcinoma. Various components affect the prognosis of CRC including bowel wall invasion, lymph node metastasis, and distant metastases (Akkoca et al. 2014). Three different staging systems are used for CRC, i.e., Dukes system, TNM system, and Astler-Coller system (Akkoca et al. 2014).

The modified Dukes staging system divides CRC into stages A, B1, B2, C1, and C2.

Stage A – Cancer has begun to spread but is still limited to inner lining (mucosa).
Stage B1 – Cancer is now spread into the submucosa, but it has not yet reached the lymph node.
Stage B2 – Cancer is now spread into the muscle layer, but it has not yet reached the lymph node.
Stage C1 – Metastases through lymph node but cancer hasn't surpassed the bowel wall.
Stage C2 – Metastases through lymph node and cancer has surpassed the bowel wall.

TNM (tumor node metastasis) staging system was introduced by the American Joint Committee on Cancer (AJCC) and the International Association of Cancer (UICC) (Akkoca et al. 2014). It is now the most commonly used classification system for deciding the treatment strategy. The classification according to the *AJCC Cancer Staging Manual 8th Edition* (AJCC Cancer Staging Manual 2017) is as follows:

T = primary tumor

It denotes the spread of CRC into various layers of the bowel (Fig. 17.1). The various stages are T0 (no primary tumor), TX (primary tumor of unknown), Tis (carcinoma in situ; cancer cells only in the epithelium lining), T1 (tumor into submucosa), T2 (tumor into muscularis), T3 (tumor into subserosa), T4a (tumor into visceral peritoneum), and T4b (tumor grown into neighboring organs).

N = regional lymph node

It represents the extent of lymph nodes to which the cancer has reached. It is often written as NX (regional lymph nodes can't be evaluated), N0 (no lymph node involved), N1a (tumor cells in one lymph node), N1b (tumor cells in two to three lymph nodes), N1c (discrete tumor deposits within the lymph drainage area without identifiable lymph nodes), N2a (tumor cells in four to six lymph nodes), and N2b (tumor cells in seven or more lymph nodes).

M = metastasis

It denotes the status of the CRC invasion into other tissues or organs. It is often written as M0 (no metastasis), M1a (metastasis to one site or organ), M1b (metastasis to more than one site or organ), and M1c (metastasis to the peritoneal surface).

The stages of CRC based on TNM classification are given in Table 17.1 (AJCC Cancer Staging Manual 2017).

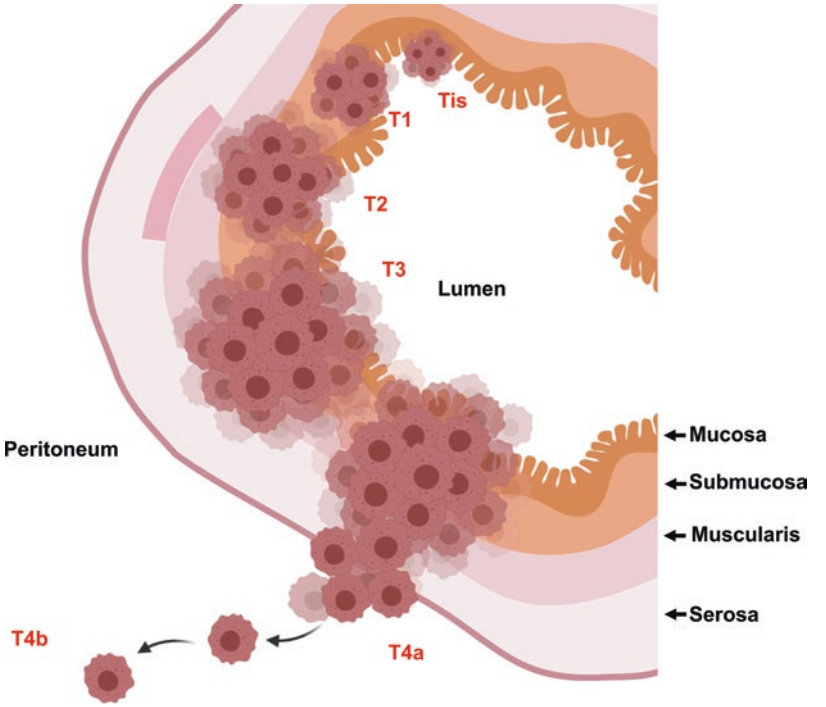

Fig. 17.1 Spread of colorectal cancer through various layers of bowel wall

Table 17.1 Stages of colorectal cancer according to AJCC manual

Stage	TNM status
0	Tis, N0, M0
I	T1/T2, N0, M0
IIA	T3, N0, M0
IIB	T4a, N0, M0
IIC	T4b, N0, M0
IIIA	T1–T2, N1/N1c, M0
	T1, N2a, M0
IIIB	T3–T4a, N1/N1c, M0
	T2–T3, N2a, M0
	T1–T2, N2b, M0
IIIC	T4a, N2a, M0
	T3–T4a, N2b, M0
	T4b, N1–N2, M0
IVA	Any T, any M, M1a
IVB	Any T, any M, M1b
IVC	Any T, any M, M1c

17.5 Role of EVs in CRC

17.5.1 EVs in Tumor Progression

The EVs extruded from cancer cells contribute significantly to the development of crucial stages through intercellular communication. In CRC also, a multistep process leads to the transformation of a healthy cell into adenocarcinoma. Cancer cell-derived EVs can impart oncogenic factors to healthy cells in the form of cargos of cell cycle-related molecules like proteins, mRNAs, miRNAs, etc. for the initiation of tumorigenesis and angiogenesis (Chiba et al. 2012; Hong et al. 2009; Melo et al. 2014; Skog et al. 2008; Tai et al. 2018). Exosomes derived from CRC cells can transport mRNAs, miRNAs, and natural antisense RNAs (asRNAs) into other cancer cells like liver and lung cancers (Chiba et al. 2012). A majority (30–40%) of the cases of CRC carry a mutation in the gene KRAS, which affects the metastasis of CRC cells to other organs like the brain and lungs (Camp and Ellis 2015). It has been found that the mutant KRAS in the CRC cells influences miRNA and proteomic profile of the exosomes extruded from these cells (Cha et al. 2015; Beckler et al. 2013). Mutant KRAS CRC cells have an enhanced miR-100 expression in exosomes, which is functional in the receiving wild-type KRAS cells (Cha et al. 2015). EVs from mutant KRAS cells harbor several oncogenic factors like KRAS, EGFR, SRC family kinases, integrins, etc. Exosomes from mutant KRAS cells can transfer these factors into the CRC cells with wild-type KRAS and trigger their 3D growth (Beckler et al. 2013).

The EVs released from colon cancer cells under hypoxic stress have been reported to induce self-proliferation and shorten the mitotic cycle (Ren et al. 2019). The hypoxic CRC cell-derived EVs could also induce the growth and movability of endothelial cells in vivo by activating Wnt/β-catenin signaling pathway (Huang and Feng 2017). Proteomic analysis of exosomes from CRC patients identified 846 proteins with implications in alteration of epithelial polarity, metastasis, tumor development, angiogenesis, and immunomodulation (Choi et al. 2007). Microarray analysis of mRNAs of healthy, adenoma, and colorectal carcinoma patient cells reported reduced expression of ALIX (ALG 2-interacting protein X) in healthy patients' cells as compared to adenoma and carcinoma (Valcz et al. 2016). ALIX is a protein that is involved in the formation of exosomes and MVBs. ALIX-positive MVB-like particles were found in carcinoma in tumor microenvironment (Valcz et al. 2016). Thus, it is clear that ALIX protein is implicated in tumor exosome and MVB formation and adenoma-carcinoma transition.

Exosomal microRNAs also play a significant role in CRC progression. MVP (major vault protein)-mediated sorting of tumor suppressor miR-193a into exosomes has been found important for colon cancer progression (Teng et al. 2017). Plasma exosomal miR-21 levels also change during cancer stages in CRC because of its role in TNM staging and liver metastasis (Tsukamoto et al. 2017). The level of miR-379, a tumor suppressor, also reduces in exosomes from CRC cells (Clancy et al. 2016).

17.5.2 Instigation of Metastasis

Tumor cells are invasive and proliferative; therefore, they can divide continuously, detach from the surface, and spread into the nearby tissues (Buda and Pignatelli 2004; Cheshomi and Matin 2018). During primary tumor formation, communication between cells and local microenvironment is required. Exosomes, as mentioned earlier, can facilitate the communication between the cells through the bioactive substances they harbor. Tumor cells secrete the EVs with oncogenic molecules inside, which can be transferred from one tumor cell to another within the primary tumor (Becker et al. 2016). A number of proteins of EVs of metastatic CRC cells are modified with O-GlcNAc (O-linked β-N-acetylglucosamine) attachment at serine and/or threonine residues (Chaiyawat et al. 2016). Studies have reported that CRC cell-derived exosomes help in metastasis by increasing proliferation, transformation, and invasiveness (Wang et al. 2015). Cancer-initiating cells (CICs) possess specific markers, which are transported in exosomes to other cells. For example, exosomes containing CD44v6 (a CIC biomarker) from CRC cells have been reported to transfer migratory and invasive capacity to the non-CICs (Wang et al. 2016). The specific mechanism may involve the regulation of additional CIC markers like Tspan8 by cooperating with proteases and integrins.

Proteomics-based studies have revealed that the exosomes emerging from primary and metastatic CRC cells are rich in various metastatic factors and signaling molecules (Ji et al. 2013). Further, the EVs derived from the serum of CRC patients are also reported to promote cellular invasion and migration (Chen et al. 2017). Human colon adenocarcinoma cell line, HCA-7, also extrudes EVs containing EGFR ligands heparin-binding EGF-like growth factor (HB-EGF) and amphiregulin that would enhance invasiveness in tumor cells (Higginbotham et al. 2011). The exosomes released from mutant *KRAS*-expressing CRC cells have also been observed to crosstalk with and transfer the mutant KRAS as well as other oncogenic factors to wild-type *KRAS*-expressing cells to influence their proliferative and migratory properties (Beckler et al. 2013). Likewise, exosomes secreted from the aggressive liver metastatic CRC cells can promote poor liver metastasis exhibiting CRC cell lines (Wang et al. 2015). Also, it has been observed that early-stage primary colorectal adenocarcinoma cells secrete exosomes, which can reprogram the normal fibroblasts to invade matrix (Rai et al. 2020). Most of the tumor cells including CRC cells are capable of giving rise to an actin-rich protrusion called invadopodium that facilitates metastasis (Liu et al. 2020; Yamaguchi 2012). Invadopodia get attached to the ECM of the cells and recruit exosomes, allowing them to release MMPs, which dissolve ECM and make invasion easier. Invadopodia also serve as a site of release of exosomes, and a synergistic control between invadopodia organization and exosome formation has been seen (Beghein et al. 2018; Hoshino et al. 2013; Mu et al. 2013). However, a direct correlation of exosomes with CRC invadopodia formation has not been reported yet. EVs are known to have the ability to associate with ECM directly and transfer degrading molecules like matrix metalloproteinases, cathepsin B, etc. to promote invasion and motility (Chen et al. 2017; Rai et al. 2019).

MicroRNAs also serve as an important component of exosomes that influences the metastatic behavior of the cancer cells. MicroRNAs like miR-135b have been found to be upregulated in CRC, whereas miR-375, miR-215, miR-378, and miR-422a were downregulated. miR-375 and miR-215 play a significant part in apoptotic pathways (Faltejskova et al. 2012; Zaharie et al. 2015). Exosomes derived from CRC have been found to be rich in mRNAs, microRNAs (miR-21, miR-192, and miR-221), and naturally occurring antisense RNAs that can be transferred to hepatoma and lung cancer cells and may regulate the genes of receiving cells (Chiba et al. 2012). miR-210 present in the EVs secreted from HCT-8 colon cancer cells are found to influence the EMT and mesenchymal-epithelial transition (MET) of neighboring cells (Bigagli et al. 2016).

17.5.3 Role in Drug Resistance

There are specific cargos in tumor-derived EVs that are instrumental in the induction or enhancement of resistance to anticancer agents in cancer cells. Exosomes can sequester the chemotherapeutic agents within intracellular vesicles and successive expulsion (Maacha et al. 2019; Shedden et al. 2003). A study on melanoma, adenocarcinoma, and lymphoma cells demonstrated the retention of drugs, viz., cisplatin, 5-fluorouracil (5-FU), and vinblastine, in vesicle-like structures, which were further degranulated into the extracellular environment (Luciani et al. 2004). Further, EVs contain drug efflux pumps like P-glycoprotein 1 (P-gp1) or multidrug resistance protein 1 (MDR1) that ease the transfer of MDR to the recipient cells (Zhang et al. 2014). This mechanism can convert a sensitive cell into a drug-resistant cell. MDR mechanisms cause chemotherapy resistance including reduction in the uptake of drugs miscible in water and a rise in energy-dependent efflux of hydrophobic drugs (Robey et al. 2018). P-gp is one of the major anticancer pumping transporters (Robey et al. 2018). Previous studies have reported that exosomes contain either the whole protein that is functional or the mRNA required for encoding the protein. In different carcinomas like prostate, ovarian, leukemia, and osteosarcoma, exosomes that carry MDR proteins can convert sensitive cells into drug-resistant ones (Bebawy et al. 2009; Corcoran et al. 2012; Torreggiani et al. 2016; Zhang et al. 2014). Thus, EVs regulate drug efflux pumps that eventually affect drug resistance.

The transfer of miRNAs, which causes alternations in EMT-mediated signaling pathways leading to an upregulation of drug efflux pumps, has been observed in ovarian cancer (Crow et al. 2017). In CRC, it has been noticed that exosomes can activate Wnt/β-catenin pathway, resulting in the resistance toward 5-FU and oxaliplatin (Hu et al. 2019). Co-culturing of sensitive CRC cells with oxaliplatin-resistant CRC cells could transfer miR-46146 in an exosome-dependent way to induce drug resistance in sensitive cells (Xu and Zhu 2020). Exosomes released from cetuximab-resistant RKO colon cancer cell line have also been reported to induce cetuximab resistance in cetuximab-sensitive Caco-2 cells by modulating the PTEN

(phosphatase and tensin homolog) and phospho-Akt pathway (Zhang et al. 2018). Exosomal transfer of p-STAT3 (phospho-signal transducer and activator of transcription 3) also develops resistance toward 5-FU in CRC cells (Zhang et al. 2019). CRC cell-derived miRNA like miRNA-200 family members in exosomes suppressed EMT-regulating transcription factors. The absence of miR-200c, miR-141, and miR-429 in 5-FU-resistant carcinoma cells can sensitize LECs (lymph endothelial cells) to the migratory signal and accelerated CCID (circular chemorepellent-induced defect) formation in BEC (brain endothelial cell) barrier (Holzner et al. 2016).

It is studied that the tumor-derived EVs have the ability to induce drug resistance by targeting apoptosis regulators. For example, downregulation of PTEN and enhanced phosphorylation of Akt can induce cetuximab resistance in sensitive cells by receiving exosomes from resistant CRC cells (Zhang et al. 2018). Moreover, exosomes derived from metastatic CRC cells containing long noncoding RNA (lncRNA) urothelial carcinoma-associated 1 (UCA1) have been found to transfer cetuximab resistance to sensitive cells (Yang et al. 2018). Exosomes isolated from carcinoma-associated fibroblasts (CAFs) can also introduce chemoresistance in CRC stem cells (Hu et al. 2015).

17.5.4 Tumor Diagnostic and Prognostic Biomarker

It is very important to detect CRC at an initial stage to start the treatment earlier. In the past few years, the potential of the EVs as a prognostic and diagnostic biomarker based on their cargos has been studied. The cargos can be a variety of RNAs, viz., miRNAs, lncRNA, circular RNAs, and proteins. The antigens present on the surface of EVs can help in the identification of the organ of origin (Mousavi et al. 2019). The most studied ones are miRNAs. As miRNAs are easily available in most of the exosomes in the body fluid, it becomes easier to isolate them and study (Cheshomi and Matin 2018; Xiao et al. 2020). miRNAs in EVs are covered by a membrane, and hence they remain protected from RNAses (Koga et al. 2011). They are stable at room temperature for many days and are easily detectable (Mousavi et al. 2019). miR-21 is expressed highly in CRC, but it is not a specific biomarker because of being expressed in other types of cancers too (Mousavi et al. 2019). A study recognized 16 miRNAs of EVs of colorectal cancer origin as diagnostic biomarkers, namely, miR-1915, miR-1308, miR-1290, miR-1268, miR-1246, miR-1229, miR-1224, miR-638, miR-483-5p, miR-223, miR-181d, miR-181b, miR-150, miR-23a, miR-21, and let-7a (Ogata-Kawata et al. 2014). Among these, miR-1246 and miR-23a demonstrated 95.5% and 92.0% sensitivity, respectively, which is much higher than other known biomarkers of CRC. A set of seven miRNAs (i.e., let-7a, miR-1229, miR-1246, miR-150, miR-21, miR-223, and miR-23a) was evaluated and reported as a suitable biomarker for CRC (Ogata-Kawata et al. 2014). Exosomes with miR-100, miR-200, miR-223, miR-1229, miR-1224-5p, and let-7a also possess diagnostic value (Cheshomi and Matin 2018). Other studies have

emphasized the use of miR-18, miR-1229, let-7a, miR-150, miR-1246, miR-223, miR-21, and miR-23a for the diagnosis of CRC (Cheshomi and Matin 2018; Komatsu et al. 2014). The EVs present in the serum of CRC patients have been found rich in miR-125a-3p, miR-19a, and miR-4772-3p and can be used as a prognostic marker (Liu et al. 2016a; Zhao et al. 2017a). Other than these, miR-193a and miR-21 also can be used as a prognostic biomarker in the patients of CRC (Cheshomi and Matin 2018; Zhao et al. 2017b). It has also been observed that the cells secreting the exosomes with a lowered expression of miR-200 have more tendency to metastasize into the blood and lymphatic system (Senfter et al. 2015).

The CRC-derived EVs are rich in carcinoembryonic antigen (CEA) and epithelial cell adhesion molecule (EpCAM) that are important markers of tumor (Dai et al. 2005; Tauro et al. 2013a). Other protein biomarkers exhibiting on the surface of CRC-EVs are vaccinia virus antigen A33, EGFR, mitogen-activated protein kinase 4, proliferating cell nuclear antigen (PCNA), and keratin 18 (Mathivanan et al. 2010). Proteomic evaluation of EVs secreted for the ascites from colon cancer cell line has shown the presence of markers like ephrin-B1 and cadherin-17 (Choi et al. 2007; Mathivanan et al. 2010). CRMP-2 (collapsin response mediator protein-2) has also been observed in colon adenocarcinoma cell lines and eventually confirmed in the serum of the patients with CRC, suggesting it to be a potential biomarker (Wu et al. 2008). CRMP-2-positive rate was higher in early-stage cancer and lymph node metastasis. Another study has identified exosomal glypican 1 (GPC1) as a diagnostic and post-surgery marker in the plasma of patients with CRC (Li et al. 2017a). Along with increased GPC1 exosomes, a reduced plasma level of noncoding RNAs miR-96-5p and miR-149 has also been found significant in the diagnosis of CRC.

Long coding RNAs (lncRNAs) released by exosomes also have been studied. It has been reported that exosomal lncRNAs like UCA1, H19, and upregulated CRNDE-h (colorectal neoplasia differentially expressed-h) and zinc finger antisense 1 can be used for the diagnosis of CRC and other cancers (Bian et al. 2016; Cheshomi and Matin 2018; Han et al. 2016; Han et al. 2017; Hashad et al. 2016; Liu et al. 2016b; Wang and Xing 2016). Bioinformatic analysis documented that bladder cancer-associated transcript 1 (BLACAT1) and other downregulated lncRNAs (LOC344887, LINC00675, DPP10-AS1, HAGLR) are biomarkers of CRC (Dai et al. 2017). Exosomal BCAR4 lncRNA in the serum of colon adenoma patients was identified as a biomarker of CRC along with two mRNAs, i.e., KRTAP5-4 and MAGEA (Dong et al. 2016). A clinical trial (NCT04394572) has been started to find novel protein biomarkers on exosomes derived from CRC cells in patients' serum.

17.5.5 EVs in the Regulation of Immune Response to Cancer

Immunosuppression is critical for tumor development. Tumors developing in intestinal epithelium also escape the immune system of the patient by various mechanisms. Recently, it is come to know that the tumor-derived EVs can also modify the tumor microenvironment by inducing immunosuppressor cells and repressing the

immune effector cell response (Corrado et al. 2014; Hellwinkel et al. 2016; Syn et al. 2016). EVs can control the activation of CD4+ and CD8+ T cells through antigen presentation (Bobrie et al. 2011; Whiteside 2016). Exosomes from CRC have been reported to induce cancer-like structural and functional changes in colonic mesenchymal stromal cells (cMSCs), thus modifying the tumor-stromal interactions (Lugini et al. 2016). This modification leads to immunosuppression due to the formation of acidic environment and spheroids (Siveen et al. 2019). Exosomes from CRC can deliver immunosuppressive and apoptotic signals to CD8+ T cells, viz., FasL and TRAIL (Huber et al. 2005). Exosomes enriched in FasL and TRAIL have been extracted from the plasma of CRC patients too. CRC cells also release EVs loaded with miR-424 and miR-503 that can regulate the expression of CD28 (Subramanian et al. 2019). Exosomes from several types of cancers including CRC have been reported to be enriched in CD39 and CD73 ectonucleotidases, which are known to convert ATP into adenosine (Allard et al. 2017; Clayton et al. 2011). Extracellular adenosine can negatively control T-cell responses (Passarelli et al. 2019). EVs released from CRC cells also regulate the differentiation of monocytes into dendritic cells/macrophages (Baj-Krzyworzeka et al. 2016; Valenti et al. 2007). The tumor-derived exosomes in the serum and tissue of CRC patients were documented to be more enriched with miR-196b-5p than healthy control. Also, it has been noted the patient with high miR-196b-5p had low survival rate, and this is connected with the targeting of immune regulators, SOCS1 and SOCS3, of STAT3 signaling pathway that led to activation of the STAT3 signaling and hence promoted stemness and chemoresistance against 5-FU (Ren et al. 2017). However, on the contrary to immunosuppression activity, heat shock protein 70 (Hsp70) has also been identified in the CRC-derived exosomes, which can induce motility and cytolytic activity in natural killer cells (Asea et al. 2000; Gastpar et al. 2005).

17.5.6 EVs in Cancer Therapeutics

EVs have many properties that make them suitable for use as a drug delivery vehicle, viz., antigen presentation (Lindenbergh and Stoorvogel 2018; Smith et al. 2017), easily crossing biological barriers (El Andaloussi et al. 2013; Yang et al. 2015), non-toxic, non-immunogenic, and easy to manipulate (Villa et al. 2019). EVs can be administered through different physiological routes and loaded with a variety of cargos like RNA, proteins, and drug molecules. They can protect their cargo molecules from the enzymatic and non-enzymatic degradation and are stable in biological fluids (Murphy et al. 2019; Sil et al. 2019). Due to nanoparticle size, they can avoid phagocytosis by mononuclear phagocytes (van den Boorn et al. 2011). All these properties make exosomes an excellent drug delivery vehicle. It is documented that EVs, if bioengineered to harbor miR-379 and transferred to recipient cells, can retard the growth and movement of CRC cells (Clancy et al. 2016). The exosomes loaded with doxorubicin and coated with A33 antibodies have shown antiproliferative activity against A33-positive colorectal cells (Li et al. 2018). Tumor-derived

exosomes can also act as a vaccine. In a study, heat stress was induced in CEA-positive tumor cells to produce heat shock protein and major histocompatibility complex-1 (MHC-I) to increase the immunogenicity of released exosomes. Immunization of HLA-A2.1/Kb transgenic mice with these exosomes could produce CEA-specific anti-tumor activity (Dai et al. 2005). A phase I clinical trial (NCT01294072) is under investigation for the efficacy of plant exosomes in delivering curcumin to colon tumors (https://clinicaltrials.gov/). Also, a previous phase I clinical trial examined the combination of ascites-derived exosomes (Aex) with granulocyte-macrophage colony-stimulating factor (GM-CSF) for the immunization therapy of CRC (Dai et al. 2008). The therapy was found effective and safe. A MVP is overexpressed in multiple drug-resistant cancer cells. It forms a complex with miR-193a, a tumor suppressor microRNA, in the exosomes. In a study, knockout of MVP in colon cancer cells could induce accumulation of miR-193a in the cell, leading to inhibition of tumor progression in a Caprin1-dependent way (Teng et al. 2017).

17.6 Conclusion

EVs have diagnostic and prognostic value in cancer progression and metastasis due to their various properties like cell specificity, sensitivity, stability, crossing biological barriers, and easy isolation. But it is difficult to process due to some limitations, even though many EV-/exosome-based diagnostic kits are approved by the Food and Drug Administration (FDA) and used in clinical diagnosis. The limitations include time-consuming and costly isolation process, lack of purity, false results due to contamination of other RNAs/proteins, and heterogeneity. Still, EVs can be used widely because of the advantages they offer. EVs can be used to make cancer vaccines as well as can be loaded with chemotherapeutic agents for targeted delivery to specific cells. A number of clinical trials are going on for the evaluation of EVs as a biomarker for the diagnosis/prognosis of cancer and therapeutics. However, the use of EVs is still under investigation, and easier methods are required for their characterization. The therapeutic and diagnostic potential of EVs in vivo needs to be studied in detail.

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Chapter 18
Probiotics in Colon Cancer: A Therapeutic Approach

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Abstract Normal microflora is the most essential and key player in maintaining good health. When the focus is on the digestive system, it is the microflora and the enzymes that co-ordinate to carry out the functions properly. Since gut microbiota and colon cancer are closely linked, many studies are being carried out to evaluate the impact of probiotics on the pathology of the disease. The literature suggests various mechanistic roles of the probiotics like decreasing the incidence of diarrhoea, inflammatory bowel disease, etc. Although adopting probiotics as mainline treatment needs clinically relevant analysis and deeper studies, they can be prescribed as a daily dose to reverse the effect of chemotherapy and radiotherapy. This chapter will therefore focus on the most recent research on use of probiotics in treating colon cancer, their strategy, efficacy pertaining to the stage of cancer and treatment mode.

Keywords Probiotics · Gut microbiota · Colorectal cancer

18.1 Introduction

The human microflora has been evolved to function at its best in order to maintain homeostasis. As the colon and rectum belong to the gut, most of its physiological activity relies on how healthy the gut microbiota is. Hence, occurrence of colorectal cancer (CRC) is largely influenced by diet, while its treatment and recovery can be enhanced by suitable probiotics. According to the World Cancer Research Fund, CRC is the third most common cancer owing to the lifestyle change reflected in food which mainly consists of processed as well as red meat, together with alcohol consumption and smoking habits (Rawla et al. 2019). This is evident from a recent study which reports an increased incidence of CRC mainly from high-income coun-

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tries (Araghi et al. 2019). Since diet is the most easily modifiable aspect here, inclusion of dietary fibres and phytochemicals will enhance the gut microbiota (Tao et al. 2020). However, for CRC patients, intake of probiotics could be the simplest and most efficacious way to improve survival rate as well as to curb the suffering and revival of the disease. The probiotics are defined as "Live micro-organisms when administered in adequate amounts, confer a health benefit on the host" (Hill et al. 2014). Cancer treatment methodologies has received much attention and technology inputs but cases of recurrence remain high. This is mainly because post-treatment strategies are not given enough focus they deserve. As far as CRC is concerned, limited studies exist on established facts regarding the use of probiotics, but some studies have proved that probiotics reduce the extent of symptoms like diarrhoea, inflammatory bowel disease, etc. In this chapter, we will look into the various probiotic species tested to use and their mechanism of action based on the recent studies.

The two sections of microorganisms involved throughout the discussion will be (i) the intestinal microflora which largely consists of *Actinobacteria*, *Firmicutes*, *Proteobacteria* and *Bacteroidetes* and (ii) the probiotic strains which mostly belong to the genera of *Lactobacillus* and *Bifidobacterium*. The latter also reside in the gut to perform sugar degradation and consequent production of lactic acid. The species of these genera predominantly produce antioxidant enzymes like glutathione (GSH), catalase, superoxide dismutase (SOD), etc. which are required for maintaining homeostasis in the digestive system. In addition to this, the gut flora is responsible for the production of short-chain fatty acids (SCFAs) which are the end product of lipid and carbohydrate metabolism and play a key role in balancing redox potential. These are known to bind to the specific G-protein-coupled receptors (GPCRs) 41 and 43 as signalling ligands which are described in the later sections of this chapter.

The contradicting fact about microbes involved in the regulation of the gut is they are so diverse that changes in the microenvironment can mutate them too. Or at times, due to various reasons that expose an individual to cancer, the gut may acquire oncopathogenic microbes. As L Farhana and colleagues reviewed, the heterogeneity of the gut microbiota plays a crucial role in CRC tumour progression (Farhana et al. 2018). Hence, immense focus has to be given while engineering probiotics which could also target such selective organisms so as to prevent infections and secretion of virulent metabolites. Probiotic bacterial strains of *Lactobacillus* and *Bifidobacterium* which are commonly found in fermented milk products are among the most prominently studied genera.

18.2 Recent Findings in Newer Probiotic Strains

18.2.1 In Vitro Studies

Lactobacillus paracasei subsp. *paracasei* M5L is a strain with precise mechanism reported, and it has shown to induce apoptosis in HT-29 cells by producing reactive oxygen species (as superoxide dismutase and catalase were found to be inactive). It

can also induce S-phase arrest and ER stress by translocating calreticulin from ER (endoplasmic reticulum) to cell membrane (Hu et al. 2015). Several studies have tried to focus on the importance of the effect of exopolysaccharide as it has multiple functions. It manipulates the gut microbiota and enhances short-chain fatty acid (SCFA) production. SCFA is known to play a key role in host physiological aspects, such as supplying nutrients to cells of the colon, helping to maintain epithelial health and homeostasis and inhibiting the growth of pernicious bacteria and the occurrence of colorectal cancer. *Rhizopus nigricans* is a filamentous fungus, and from its fermentation broth, an extracellular polysaccharide was extracted. It proved to act as an excellent immunomodulant against CRC-induced mice model. It could also increase the villus length, ratio of villus length and crypt depth in colonic tissues and improve the number of acid mucus-secreting goblet cells (Yu et al. 2018). *Gordonibacter urolithinfaciens* and *Gordonibacter pamelaeae* are two organisms in the gut which are capable of converting certain ellagitannins to urolithin A. It has been proved to elicit anti-inflammatory properties and upregulate T-cell-mediated immune response (González-Sarrías et al. 2015). In another study, co-treatment of 5-fluorouracil (5-FU) and 5′DFUR with urolithin A decreased the IC_{50} doses of the former drugs and also exhibited additional mechanism of G2/M phase cell cycle arrest together with a slight increase in caspases 8 and 9 activation (Zhang et al. 2019). Bioactive compounds extracted from probiotic strains pose an effective option too. In a novel kind of study, probiotic strain *Lactobacillus paracasei* subsp. *paracasei* isolated from NTU 101-fermented reconstituted skimmed milk ethanol extract containing a mixture of palmitic acid, stearic acid and glyceryl 1,3-dipalmitate was found to effectively decrease CRC cell viability. Upregulation of apoptosis-related proteins and downregulation of NFκB were demonstrated. Co-treatment with 5-FU produced better results suggesting its potential use in adjuvant therapy (Chang and Pan 2019). Biogenics is a newly conceptualized treatment strategy against CRC wherein certain lactic acid bacterial components are extracted and formulated which exert their function irrespective of the environment of the gut. In one such study, Nobuhiro Hiraishi and colleagues prepared and tested an extract of *Lactobacillus plantarum* strain 06CC2. Basically, the species was heat-killed and powdered, and the bacterial debris was removed via filtration. This was used to treat the cancer cell lines, and apoptosis was induced via ER stress and JNK-/p38-mediated pathways (Hiraishi et al. 2019). At times, it is a metabolite or a biomolecule produced by the bacteria that possesses anti-cancer activity. For example, *Lactobacillus acidophilus* NCFM has a surface layer protein which is toxic to HT-116 cell lines. In the study conducted by Huifang Wang and associates, they showed that this surface layer protein can induce autophagy through ROS (reactive oxygen species)-mediated mTOR pathway as well as JNK pathway. Other mechanisms can be explored; however, it is the information that is of significance as it could be considered as a potential probiotic in colon cancer treatment (Wang et al. 2019a).

Lactobacillus cocktail has gained prominence due to its synergistic effect. A study by Roya Ghanavati et al. involves the use of *Lactobacillus* cocktail which is shown to induce apoptosis via modulation of *Notch* and *Wnt* signalling pathways. The main advantage of using cocktail is to avoid effects arising out of toxic metabo-

lite like butyrate or conjugated linoleic acids, which is described in the report (Ghanavati et al. 2020a). The simultaneous targeting of specific genes which together results in added benefit is an effective way of research to be adopted to find the best probiotic therapy. In a mechanism involving modulation of *hes1* pathway, the former group could also demonstrate that the cocktail could modulate Hes1 which involved downstream of both notch-dependent and notch-independent pathways (Ghanavati et al. 2020b).

18.2.2 Studies in Animal Models

Pediococcus pentosaceus GS4 was reported to possess a unique property of producing conjugated linoleic acid (CLA) via biohydrogenation. The studies involved testing the mechanisms *in vitro* and *in vivo*. In cell lines, it alleviated CRC by inducing apoptosis through NFκB and p-AKT pathways, while in azoxymethane-induced animal models, it decreased the biohydrogenation of the gut microflora and at the same time triggered apoptosis which was evident from various pathophysiologies of cancer cells, viz. DNA fragmentation, caspase 3 activity, (Poly-ADP ribose polymerase) PARP cleavage, epigenetic changes and so on (Dubey et al. 2016). *Bifidobacterium animalis* subspecies *lactis BL3* is a Korean species which has been fully sequenced after it was reported to have anti-inflammatory properties especially against colon cancer (Kang et al. 2017). R7 metabolite isolated from Ricotta cheese *Lactococcus lactis* subsp. *lactis* showed protective effect against 1,2-dimethylhydrazine (DMH)-induced CRC in animal model. It was known to enhance immune response especially pertaining to the mucosal immune system. The isolate also demonstrated hypocholesterolemic potential (Jaskulski et al. 2020). While studies concentrated on determining the mechanism of existing probiotic microorganisms, certain group focused on isolation of novel species. Meiling Liu and team evaluated a novel probiotic strain *Lactobacillus casei* (*L. casei*) LH23 for its efficacy. It could reduce inflammation in lipopolysaccharide (LPS)-induced RAW 264.7 cell lines by inhibiting overactivation of JNK/p38 pathway. Additionally, in *in vivo* studies in mouse model, it decreased production and release of inflammatory cytokines and myeloperoxidase activity. The most significant and rare benefit exhibited by LH23 is restoration of H3K9 acetylation in colon tissues (Liu et al. 2020). *Lactobacillus plantarum* strain YYC-3 is present among the gut microbiota. This along with its cell-free supernatant YYCS was demonstrated for their anticancer activity in mouse model. Reduction in the inflammatory cytokines IL-6, IL-17 F and IL-22 and enhancement of the immune system altogether were seen. YYC-3 was demonstrated to be comparatively more potent as it tried to restore the environment of the gut microbiota by downregulation of NFκB pathway and altering Wnt signalling pathway (Yue et al. 2020). Similar to biogenics, CRC, like any other type of cancer, is prone to metastasize. The liver is one of the organs vulnerable to this end, and a probiotic strain of *Lactobacillus gasseri* 505 in combination with *Cudrania tricuspidata* leaf extract is known to reduce liver cancer toxicity caused due to CRC. In azoxymethane-induced CRC animal model, synbiotic prepa-

ration of the probiotic strain and leaf extract were added to fermented milk and freeze-dried for studies. Such preparations stand a chance to be used as candidates for enhanced probiotic effects (Oh et al. 2020). G-protein-coupled receptors (GPCRs) GPCR43 and GPCR109 are known to mitigate proliferative pathways and transformation of normal human colon tissue to carcinoma. *Clostridium butyricum* was shown to reduce high-fat diet-induced CRC in mice animal model. However, the exact role and benefit of butyrate-producing bacteria in gut microbiota are debatable. Extensive studies are needed to consider their use as potent probiotics (Chen et al. 2020).

18.3 Probiotics and Colon Cancer Treatment

Probiotic treatments are based on many factors (Fig. 18.1) because their effects can be mediated by (a) manipulation of gut microbiota (Ak and To 2014; Montalban-Arques et al. 2015), (b) reduction of enzymes that convert amines and hydrocarbons to active carcinogens (Hatakka et al. 2008) or as seen in most cases (c) enhancing the immune response and finally (d) induction of apoptosis through various pathways. Enzymes like beta-glucosidase, beta-glucuronidase and urease, apart from functioning normally, transform certain food chemicals to carcinogenic agents. Hatakka et al. have shown in their studies how the combination of two strains of bacteria *Lactobacillus rhamnosus* LC705 and *Propionibacterium freudenreichii* ssp. *shermanii JS* can help in decreasing the production of these enzymes (Hatakka et al. 2008). Discussing about probiotic strains and immune response, there is no

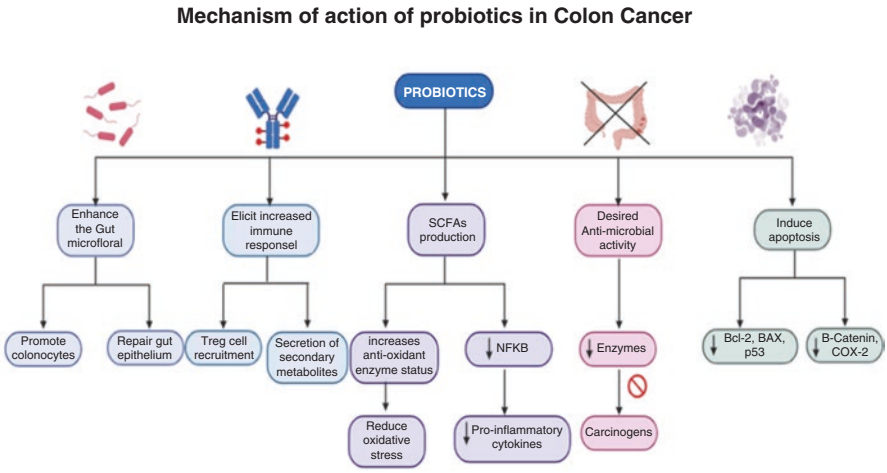

Fig. 18.1 Probiotics are mainly involved in alteration of gut microflora which in itself can carry out several functions. Apart from that, bioactive compounds from certain probiotics can be used which induces apoptosis-mediated roles

fixed pattern where we can hypothesize whether species from a particular strain can elicit an immune response. Dosage, time and individual's response to the administered probiotic play an important part. Preliminary studies as part of standardization, randomized and controlled trials, placebo trials, etc. will be required if the strain is found to be potent. Many studies focusing on inducing apoptosis have already been discussed.

18.3.1 Alteration in the Enzymatic Activity

The liver is the foremost and the major organ involved in detoxification, followed by the intestine wherein the microbiota release enzymes which deconjugate certain harmful carcinogenic agents leading to their excretion. At times, due to several reasons, there is a surge in the amount of β-glucuronidase, β-glucosidase and azoreductase enzymes, and these have been shown to turn foodborne chemicals into carcinogenic agents (Humblot et al. 2007; Dashnyam et al. 2018). The conversion to carcinogenic agents destroys the normal intestinal flora causing tumorigenesis and production of undesirable by-products. Studies have shown the consumption of probiotics can reverse the effect induced by these enzymes. Preter et al. showed that when diet consisting of lactulose and oligofructose-enriched inulin along with probiotic strain of *Lactobacillus casei Shirota*, *Bifidobacterium breve* and *Saccharomyces boulardii* was given to subjects in a randomized crossover study, a decrease in the activity of beta-glucosidase and beta-glucuronidase in the gut was observed which was beneficial for the subjects given the condition (De Preter et al. 2008). Simple diet with less of protein and more of milk products and green vegetables are suggested during such times.

18.3.2 Immunomodulation by Probiotics

Inflammation is the characteristic of most of the non-communicable diseases including cancer. Various signalling pathways can get involved in upregulating the expression of inflammatory markers and the most prominent one being k-ras as it is an oncogene. Downstream to this, all other physiological changes of cancer progression occur like angiogenesis, metastasis, etc., and in the case of colon cancer, it is inflammation that leads to damage of the intestinal epithelial lining cell junction proteins. So overall, probiotics have to exert multiple roles in order to restore the damage and enhance the immunity (Fig. 18.2). As we look into how probiotics can take part in imparting immune response, the below given figure provides a brief summary.

As the probiotic strains enter the system, they encounter T cells which activate and proliferate a large number of helper T cell into the population (Erdman and Poutahidis 2017; Goubet et al. 2018). On antigen presentation at the site of tumour, these T helper cells recruit cytotoxic T cells and establish acute immune response (Zitvogel et al. 2018; Coleman and Haller 2018).

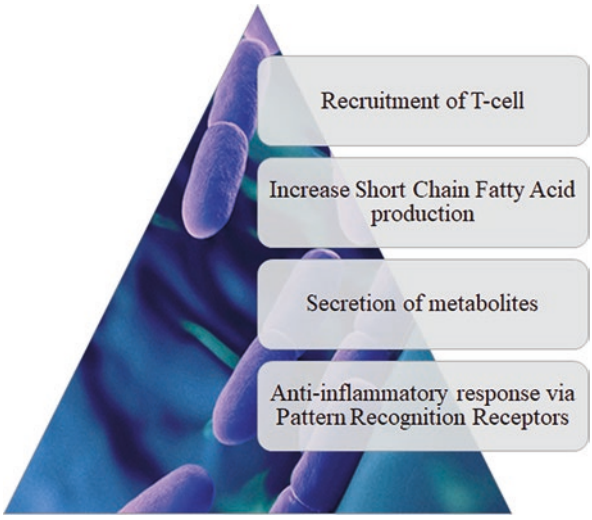

Fig. 18.2 Illustration of ways through which probiotics enhance immune response

Microbes are known to produce secondary metabolites. Since the gut is a source of the same, it has ample polyamines which have been shown to induce autophagy (Tofalo et al. 2019; Wang et al. 2019b). We have already discussed about certain metabolites and bacterial components which have immunomodulatory effects in the in vitro models. Similarly, as previously discussed, SCFAs act as ligands for dendritic cells that activate T cells. Additional effects are mitigated by SCFAs to suppress inflammation and enhance immune response:

(a) SCFAs can inhibit histone deacteylases (HDACs) which in cancer cells can suppress gene expression of NFκB.
(b) SCFAs are known to block TLR-4 receptor signalling which stops production of IL-6 and IL-12 pro-inflammatory cytokines and increases anti-inflammatory cytokine IL-10.
(c) They increase the expression of FOXp3 protein which in turn activates Treg cells.
(d) G-protein-coupled receptors (GPCRs) responsible for SCFAs are present in abundance in colonocytes which signal downstream activation of anti-inflammatory responses. It activates butyrate production through GPR109A coupled with an increase in differentiation of Treg cells. Butyrate produced by *Bifidobacterium* species also has proved to activate the immune response and also induce apoptosis (Moens et al. 2019).

It is not just the cell-mediated immunity that takes part but also innate immunity that gets activated. These bacterial components can bind to the pattern recognition receptors, namely, Toll-like receptors, present in the dendritic cell which trigger Th and CD8+ responses. They are known to secrete interferons which indirectly signal the release of anti-cancer effects. A study linked to this had shown that induction of IFN-γ activated by *Lactococcus lactis* subsp. *Cremoris* (Kosaka et al. 2012). And in

another study with probiotic-treated dendritic cells, an accumulation of IFN-γ was seen (Kosaka et al. 2012).

18.3.3 Inducing Apoptosis and Anti-proliferative Mechanism

Screening and selection of potent probiotic strain should be the prime aim in therapy against colon cancer.This is analogous to selection of druggable candidate with a definite mechanism of action against any particular disease of question. Killing the cancer cell is the goal, followed by preventing angiogenesis, metastasis, etc. Escaping the cell from apoptosis is the most common mechanism any cancerous cell would adopt, and hence, inducing apoptosis is usually the key mechanism looked for. The attractive part here is most cancer cells resort to glycolysis or what is called the "Warburg effect" for energy production which generates lactic acid. Since the diet consists of *Lactobacillus*, on inculcation of dairy products, these feed on the lactate to produce SCFAs. Underlying this mechanism, most studies have shown to discover novel strains of probiotics which can induce apoptosis which have been discussed earlier in the in vitro section.

18.3.4 Carcinogenic Agents in the Colon and Role of Probiotic Against It

As the most cited reason behind the occurrence of CRC is diet, it is important to understand how carcinogenic agents are getting formed in the intestine and how the microflora processes them. Consumption of processed meat as well as red meat is equivalent to intake of certain carcinogens. Heating or processing of the meat can lead to the production of the following:

(i) Heterocyclic amines (HCAs), such as amino-3,4-dimethylimidazo quinolone (MeIQ) and 2-amino-3,8-dimethylimidazo quinoxaline (MeIQx)
(ii) N-nitroso compounds (NOCs), such as N-nitrosodimethylamine (NDMA)
(iii) Polycyclic aromatic hydrocarbons (PAHs), such as benzo[a]pyrene (BaP)
(iv) N-glycolylneuraminic acid (Neu5Gc)
(v) High amount of bile acid due to intake of such a high-fat/protein diet that can destroy the mucosa and the colonic epithelium in the long run

Lactobacillus has been reviewed to have tremendous potential in counteracting the carcinogenic agents that are formed. The binding capacity of either whole cells, cell wall skeleton (CWS) or any component of CWS of *Lactobacillus acidophilus* IFO 13951 and *Bifidobacterium bifidum* IFO 14252 to six HCAs (Trp-P1, Glu-P-1, Phe-P-1, MeIQ, IQ and MeIQx) was examined. The potential to bind varied among the strains for different mutagens tested. The binding of Trp-P-1 and Trp-P-2 was the highest, but the binding of Glu-P-1, Phe-P-1 and IQ was lower by the two bacteria.

Treating whole cells and CWS by lysozyme and α-amylase decreased the binding of Trp-P-1 and Trp-P-2 by about 30%. The authors clearly revealed that the binding activity of these bacteria was due to the peptidoglycan of (Cell Wall Skeleton) CWS (Zhang and Ohta 1991; Khorshidian et al. 2016). In another study, binding ability of mutagens Trp-P1, PhIP, IQ and MeIQx to eight human intestinal or LAB strains (*L. acidophilus* NCFB 174, *Lactobacillus fermentum* KLD and *Bifidobacterium longum* BB 5368, *Clostridium perfringens* ATCC 1314, *Bacteroides fragilis* NCTC 9343, *Escherichia coli* ATCC 25922, *Lactococcus lactis* ssp. *lactis* NCFB 604 and *Lactococcuslactis* ssp. *Cremoris* NCFB 607) was reported (Khorshidian et al. 2016; Orrhage et al. 1994).

PAHs and HCAs interact with the DNA to form adducts in the epithelial cells of the intestine. Haeme is formed due to lipid peroxidation and also from nitroso compounds which are known to cause DNA mutations. This also leads to inflammation altogether aggregating and enhancing tumorigenesis in the colon (Cascella et al. 2018).

Foodborne infections are inevitable, so are the consequences arising out of them. These infectious microbes after entering the host can either lead to dysbiosis or production of chemicals that will induce inflammation progressing to cancerous lesion in the epithelium or create an environment suitable for growth and proliferation of pathogenic strains like *Helicobacter pylori* and so on.

These result in imbalance of the redox status, dysbiosis and inflammatory bowel diseases. Probiotics can efficiently restore the same as certain *Lactobacillus* strains have antioxidant mechanism, and as seen before, many novel strains can reduce diarrhoea by reducing inflammation and altering the gut microbiota.

18.4 Establishment of In Vitro Model Systems to Evaluate Efficacy of Probiotic Strains

The very need of having in vitro systems for analysis of the probiotic arises from the constraints being faced in in vivo models. In vivo models involve risks like obtaining ethical clearance and increased costs, variability in results, prolonged duration of experiments, etc. The advantages of in vitro system over this are the cost-effectiveness, simplicity in designing and maintaining the system, ease in obtaining results and most importantly ability to separately study the metabolism of normal flora and probiotic strain of interest (isolated or novel) (Macfarlane and Macfarlane 2007).

In vitro fermentation models vary from a simple batch system to more complex systems of continuous flow. In vitro gut fermentation models help to culture the entire gut microflora for a specific period of time which depends on the model we aim to study. It is essential to know about all the systems available – their benefits and limitations. Choosing the system for our study would again depend on our aims

and objective. The most common ones include batch, continuous, multistage continuous, stationary and continuous artificial digestive system (Zhang and Ohta 1991).

Batch fermentation involves culturing of either pure or mixed bacterial suspension in a selected medium without requiring to add any additional nutrients. These consist of sealed reactors containing faecal material. Several studies have concluded that faecal materials are an excellent source of novel species. This culture system makes it easier to obtain the metabolic profile of the gut microflora based on how they digest the dietary components.

Continuous culturing demands intense monitoring of most of the significant parameters of the digestive system right from pH, temperature, oxygen levels, etc. An undisturbed condition creates reproducible system to study metabolic activity of a large number of microbes (Rycroft et al. 2001; Pereira et al. 2003). These models have several advantages, such as the ease of use of the system, the possibility of using radioactive substances and the low operation cost. Continuous culture fermentation models consist of either single- or multistage systems and are perfect models to perform long-term studies, as substrate replenishment and end product removal, if toxic, are facilitated. Single stage continuous fermation models consists of the flora digest from both the caecum and ascending colon and hence it is suitable for studying proximal colon functions (Sivieri et al. 2011).

The multistage continuous fermentation is to date the most advanced system to demonstrate more than two common parameters that associate with physiological functions of the colon. TNO gastrointestinal models (TIM) are the computer-controlled multi-compartmental systems resembling the human digestive tract, of which TIM-1 includes parameters of the small intestine like pH, motility, bile secretion and absorption, whereas TIM-2 includes peristaltic movement patterns and absorption of nutrients which are the function of the proximal colon. When TIM-1 and TIM-2 are combined, it becomes an ideal artificial system to investigate probiotic efficacy, formulation and standardized dosages and time period. It thus becomes a very advanced area for drug delivery and probiotic-prebiotic studies with respect to colon cancer and other associated diseases (Payne et al. 2012).

18.5 Probiotics as Adjuvants

As the efficacy of probiotics gains momentum, another question that needs to be addressed is “in what form?” Consumption in diet is a matter that has been left to food technologists who are working upon bringing palatable products into the market, but this can heal basic illnesses probably and not act as a therapeutic approach. Formulations or adjuvants are a debatable topic, and we present evidence and discussions on this. Clinical trials are ongoing, and based on the efficacy and taking into consideration whether the balance falls on the benefits or risks, it will be easier to determine this. Factors like dosage, immune response on consumption of probiotics, etc., have to be considered before administration. Table 18.1 gives the latest report of ongoing clinical trials.

Table 18.1 Probiotics that have completed the clinical trials

Interventions	Title	Status
Dietary supplement: probiotic	An Evaluation of Probiotic in the Clinical Course of Patients with Colorectal Cancer	Completed
Procedure: probiotics (La1, BB536) Biological: probiotics (La1, BB536)	Probiotics in Colorectal Cancer Patients	Completed
Dietary supplement: *probiotic formula*	Prevention of Irinotecan-Induced Diarrhea by Probiotics	Completed
Dietary supplement: *Saccharomyces boulardii*	Impact of Probiotics in Modulation of Intestinal Microbiota	Completed
Dietary supplement: *Saccharomyces boulardii*	Impact of Probiotics on the Intestinal Microbiota	Completed
Dietary supplement: synbiotics	Synbiotics and Gastrointestinal Function Related Quality of Life After Colectomy for Cancer	Completed

Out of 13 registered clinical trials as obtained from www.clinicaltrials.gov, only the studies listed above are completed, and 4 are currently ongoing, 1 is to be started, and the status of 2 studies is unknown

This suggests that on adverse cases consumption of probiotic is definitely beneficial, and they stand a chance to take hold of the market as an adjuvant for CRC therapy along with other standard drugs which is also evident from studies as previously discussed (Chang and Pan 2019). There cannot be a lesser expensive and lesser painful procedure to replace this attractive treatment approach. Even recent report including randomized double-blind placebo controlled trial has shown use of *Lactobacillus* and *Bifidobacterium* to be safe for consumption after surgery (Zaharuddin et al. 2019).

18.6 Conclusion

Thus, the review draws us to a conclusion that probiotics can enhance recovery post-surgery in CRC patients. *Lactobacillus* and *Bifidobacterium* dominate as potential strains, mimicking the gut microflora. Such studies conducted in humans also have entered the clinical trials. It is advisable to still keep the focus on mainstreaming probiotics in treating CRC, as there are fewer negative impacts of the same. Firstly, there are less reports on such studies being carried on to this end, and secondly, there is a lack of precise mode of action or mimicking of exact mechanism as demonstrated or predicted from in vitro results. These and other facts make this area much attention-seeking and a beginning to healthy start for effortless therapeutic strategy. One thing the authors suggest to include in the human studies as a parameter would be associated lifestyle-derived activities like physical fitness, alcohol consumption, smoking, diet and environmental exposure to hazardous chemicals as we see these are crucial risk factors involved in carcinogenesis and which need to be addressed during treatment and recovery as well.

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